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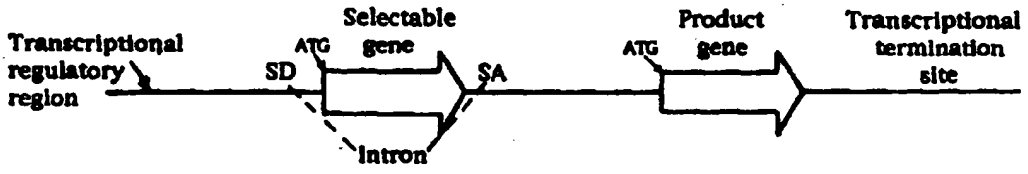

(57) Abstract

A method for selecting recombinant host cells expressing high levels of a desired protein is described. This method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified.

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METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of selecting for high-expressing host cells, a method of producing a protein of interest in high yields and a method of producing eukaryotic cells having multiple copies of a sequence encoding a protein of interest.

Description of Background and Related Art

The discovery of methods for introducing DNA into living host cells in a functional form has provided the key to understanding many fundamental biological processes, and has made possible the production of important proteins and other molecules in commercially useful quantities.

Despite the general success of such gene transfer methods, several common problems exist that may limit the efficiency with which a gene encoding a desired protein can be introduced into and expressed in a host cell. One problem is knowing when the gene has been successfully transferred into recipient cells. A second problem is distinguishing between those cells that contain the gene and those that have survived the transfer procedures but do not contain the gene. A third problem is identifying and isolating those cells that contain the gene and that are expressing high levels of the protein encoded by the gene.

In general, the known methods for introducing genes into eukaryotic cells tend to be highly inefficient. Of the cells in a given culture, only a small proportion take up and express exogenously added DNA, and an even smaller proportion stably maintain that DNA.

Identification of those cells that have incorporated a product gene encoding a desired protein typically is achieved by introducing into the same cells another gene, commonly referred to as a selectable gene, that encodes a selectable marker. A selectable marker is a protein that is necessary for the growth or survival of a host cell under the particular culture conditions chosen, such as an enzyme that confers resistance to an antibiotic or other drug, or an enzyme that compensates for a metabolic or catabolic defect in the host cell. For example, selectable genes commonly used with eukaryotic cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), neomycin, puromycin, glutamine synthetase, and asparagine synthetase.

The method of identifying a host cell that has incorporated one gene on the basis of expression by the host cell of a second incorporated gene encoding a selectable marker is referred to as cotransfectation (or cotransfection). In that method, a gene encoding a desired polypeptide and a selection gene typically are introduced into the host cell simultaneously, although they may be introduced sequentially. In the case of simultaneous cotransfectation, the gene encoding the desired polypeptide

and the selectable gene may be present on a single DNA molecule or on separate DNA molecules prior to being introduced into the host cells. Wigler *et al.*, Cell, 16:777 (1979). Cells that have incorporated the gene encoding the desired polypeptide then are identified or isolated by culturing the cells under conditions that preferentially allow for the growth or survival of those cells that synthesize the selectable marker encoded by the selectable gene.

The level of expression of a gene introduced into a eukaryotic host cell depends on multiple factors, including gene copy number, efficiency of transcription, messenger RNA (mRNA) processing, stability, and translation efficiency. Accordingly, high level expression of a desired polypeptide typically will involve optimizing one or more of those factors.

For example, the level of protein production may be increased by covalently joining the coding sequence of the gene to a "strong" promoter or enhancer that will give high levels of transcription. Promoters and enhancers are nucleotide sequences that interact specifically with proteins in a host cell that are involved in transcription. Kriegler, Meth. Enzymol., 185:512 (1990); Maniatis *et al.*, Science, 236:1237 (1987). Promoters are located upstream of the coding sequence of a gene and facilitate transcription of the gene by RNA polymerase. Among the eukaryotic promoters that have been identified as strong promoters for high-level expression are the SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Rous sarcoma virus long terminal repeat, and human cytomegalovirus immediate early promoter (CMV).

Enhancers stimulate transcription from a linked promoter. Unlike promoters, enhancers are active when placed downstream from the transcription initiation site or at considerable distances from the promoter, although in practice enhancers may overlap physically and functionally with promoters. For example, all of the strong promoters listed above also contain strong enhancers. Bendig, Genetic Engineering, 7:91 (Academic Press, 1988).

The level of protein production also may be increased by increasing the gene copy number in the host cell. One method for obtaining high gene copy number is to directly introduce into the host cell multiple copies of the gene, for example, by using a large molar excess of the product gene relative to the selectable gene during cotransfectation. Kaufman, Meth. Enzymol., 185:537 (1990). With this method, however, only a small proportion of the cotransfected cells will contain the product gene at high copy number. Furthermore, because no generally applicable, convenient method exists for distinguishing such cells from the majority of cells that contain fewer copies of the product gene, laborious and time-consuming screening methods typically are required to identify the desired high-copy number transfectants.

Another method for obtaining high gene copy number involves cloning the gene in a vector that is capable of replicating autonomously in the host cell. Examples of such vectors include mammalian expression vectors

derived from Epstein-Barr virus or bovine papilloma virus, and yeast 2-micron plasmid vectors. Stephens & Hentschel, Biochem. J., 248:1 (1987); Yates *et al.*, Nature, 313:812 (1985); Beggs, Genetic Engineering, 2:175 (Academic Press, 1981).

Yet another method for obtaining high gene copy number involves gene amplification in the host cell. Gene amplification occurs naturally in eukaryotic cells at a relatively low frequency. Schimke, J. Biol. Chem., 263:5989 (1988). However, gene amplification also may be induced, or at least selected for, by exposing host cells to appropriate selective pressure. For example, in many cases it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the cotransfected cells to sequentially increasing concentrations of a selective agent. Typically the product gene will be coamplified with the marker gene under such conditions.

The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate (Mtx). A host cell is cotransfected with a product gene encoding a desired protein and a DHFR gene, and transfectants are identified by first culturing the cells in culture medium that contains Mtx. A suitable host cell when a wild-type DHFR gene is used is the Chinese Hamster Ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub & Chasin, Proc. Nat. Acad. Sci. USA, 77:4216 (1980). The transfected cells then are exposed to successively higher amounts of Mtx. This leads to the synthesis of multiple copies of the DHFR gene, and concomitantly, multiple copies of the product gene. Schimke, J. Biol. Chem., 263:5989 (1988); Axel *et al.*, U.S. Patent No. 4,399,216; Axel *et al.*, U.S. Patent No. 4,634,665. Other references directed to co-transfection of a gene together with a genetic marker that allows for selection and subsequent amplification include Kaufman in Genetic Engineering, ed. J. Setlow (Plenum Press, New York), Vol. 9 (1987); Kaufman and Sharp, J. Mol. Biol., 159:601 (1982); Ringold *et al.*, J. Mol. Appl. Genet., 1:165-175 (1981); Kaufman *et al.*, Mol. Cell Biol., 5:1750-1759 (1985); Kaetzel and Nilson, J. Biol. Chem., 263:6244-6251 (1988); Hung *et al.*, Proc. Natl. Acad. Sci. USA, 83:261-264 (1986); Kaufman *et al.*, EMBO J., 6:87-93 (1987); Johnston and Kucey, Science, 242:1551-1554 (1988); Urlaub *et al.*, Cell, 33:405-412 (1983).

To extend the DHFR amplification method to other cell types, a mutant DHFR gene that encodes a protein with reduced sensitivity to methotrexate may be used in conjunction with host cells that contain normal numbers of an endogenous wild-type DHFR gene. Simonsen and Levinson, Proc. Natl. Acad. Sci. USA, 80:2495 (1983); Wigler *et al.*, Proc. Natl. Acad. Sci. USA, 77:3567-3570 (1980); Haber and Schimke, Somatic Cell Genetics, 8:499-508 (1982).

Alternatively, host cells may be co-transfected with the product gene, a DHFR gene, and a dominant selectable gene, such as a neo^r gene. Kim

and Wold, Cell, 42:129 (1985); Capon *et al.*, U.S. Pat. No. 4,965,199. Transfectants are identified by first culturing the cells in culture medium containing neomycin (or the related drug G418), and the transfectants so identified then are selected for amplification of the DHFR gene and the product gene by exposure to successively increasing amounts of Mtx.

As will be appreciated from this discussion, the selection of recombinant host cells that express high levels of a desired protein generally is a multi-step process. In the first step, initial transfectants are selected that have incorporated the product gene and the selectable gene. In subsequent steps, the initial transfectants are subject to further selection for high-level expression of the selectable gene and then random screening for high-level expression of the product gene. To identify cells expressing high levels of the desired protein, typically one must screen large numbers of transfectants. The majority of transfectants produce less than maximal levels of the desired protein. Further, Mtx resistance in DHFR transformants is at least partially conferred by varying degrees of gene amplification. Schimke, Cell, 37:705-713 (1984). The inadequacies of co-expression of the non-selected gene have been reported by Wold *et al.*, Proc. Natl. Acad. Sci. USA, 76:5684-5688 (1979). Instability of the amplified DNA is reported by Kaufman and Schimke, Mol. Cell Biol., 1:1069-1076 (1981); Haber and Schimke, Cell, 26:355-362 (1981); and Fedespiel *et al.*, J. Biol. Chem., 259:9127-9140 (1984).

Several methods have been described for directly selecting such recombinant host cells in a single step. One strategy involves co-transfecting host cells with a product gene and a DHFR gene, and selecting those cells that express high levels of DHFR by directly culturing in medium containing a high concentration of Mtx. Many of the cells selected in that manner also express the co-transfected product gene at high levels. Page and Sydenham, Bio/Technology, 9:64 (1991). This method for single-step selection suffers from certain drawbacks that limit its usefulness. High-expressing cells obtained by direct culturing in medium containing a high level of a selection agent may have poor growth and stability characteristics, thus limiting their usefulness for long-term production processes. Page and Snyderman, Bio/Technology, 9:64 (1991). Single-step selection for high-level resistance to Mtx may produce cells with an altered, Mtx-resistant DHFR enzyme, or cells that have altered Mtx transport properties, rather than cells containing amplified genes. Haber *et al.*, J. Biol. Chem., 256:9501 (1981); Assaraf and Schimke, Proc. Natl. Acad. Sci. USA, 84:7154 (1987).

Another method involves the use of polycistronic mRNA expression vectors containing a product gene at the 5' end of the transcribed region and a selectable gene at the 3' end. Because translation of the selectable gene at the 3' end of the polycistronic mRNA is inefficient, such vectors exhibit preferential translation of the product gene and require high levels of polycistronic mRNA to survive selection. Kaufman, Meth.

Enzymol., 185:487 (1990); Kaufman, Meth. Enzymol., 185:537 (1990); Kaufman *et al.*, EMBO J., 6:187 (1987). Accordingly, cells expressing high levels of the desired protein product may be obtained in a single step by culturing the initial transfectants in medium containing a selection agent appropriate for use with the particular selectable gene. However, the utility of these vectors is variable because of the unpredictable influence of the upstream product reading frame on selectable marker translation and because the upstream reading frame sometimes becomes deleted during methotrexate amplification (Kaufman *et al.*, J. Mol. Biol., 159:601-621 [1982]; Levinson, Methods in Enzymology, San Diego: Academic Press, Inc. [1990]). Later vectors incorporated an internal translation initiation site derived from members of the picornavirus family which is positioned between the product gene and the selectable gene (Pelletier *et al.*, Nature, 334:320 [1988]; Jang *et al.*, J. Virol., 63:1651 [1989]).

A third method for single-step selection involves use of a DNA construct with a selectable gene containing an intron within which is located a gene encoding the protein of interest. See U.S. Patent No. 5,043,270 and Abrams *et al.*, J. Biol. Chem., 264(24): 14016-14021 (1989). In yet another single-step selection method, host cells are co-transfected with an intron-modified selectable gene and a gene encoding the protein of interest. See WO 92/17566, published October 15, 1992. The intron-modified gene is prepared by inserting into the transcribed region of a selectable gene an intron of such length that the intron is correctly spliced from the corresponding mRNA precursor at low efficiency, so that the amount of selectable marker produced from the intron-modified selectable gene is substantially less than that produced from the starting selectable gene. These vectors help to insure the integrity of the integrated DNA construct, but transcriptional linkage is not achieved as selectable gene and the protein gene are driven by separate promoters.

Other mammalian expression vectors that have single transcription units have been described. Retroviral vectors have been constructed (Cepko *et al.*, Cell, 37:1053-1062 [1984]) in which a cDNA is inserted between the endogenous Moloney murine leukemia virus (M-MuLV) splice donor and splice acceptor sites which are followed by a neomycin resistance gene. This vector has been used to express a variety of gene products following retroviral infection of several cell types.

With the above drawbacks in mind, it is one object of the present invention to increase the level of homogeneity with regard to expression levels of stable clones transfected with a product gene of interest, by expressing a selectable marker (DHFR) and the protein of interest from a single promoter.

It is another object to provide a method for selecting stable, recombinant host cells that express high levels of a desired protein product, which method is rapid and convenient to perform, and reduces the numbers of transfected cells which need to be screened. Furthermore, it is

an object to allow high levels of single and two unit polypeptides to be rapidly generated from clones or pools of stable host cell transfectants.

It is an additional object to provide expression vectors which bias for active integration events (*i.e.* have an increased tendency to generate transformants wherein the DNA construct is inserted into a region of the genome of the host cell which results in high level expression of the product gene) and can accommodate a variety of product genes without the need for modification.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a DNA construct (DNA molecule) alternative terminology comprising a 5' transcriptional initiation site and a 3' transcriptional termination site, a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene positioned within an intron defined by a splice donor site and a splice acceptor site. The splice donor site preferably comprises an effective splice donor sequence as herein defined and thereby regulates expression of the product gene using the transcriptional regulatory region.

In another embodiment, the invention provides a method for producing a product of interest comprising culturing a eukaryotic cell which has been transfected with the DNA construct described above, so as to express the product gene and recovering the product.

In a further embodiment, the invention provides a method for producing eukaryotic cells having multiple copies of the product gene comprising transfecting eukaryotic cells with the DNA construct described above (where the selectable gene is an amplifiable gene), growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene. Preferably transfection of the cells is achieved using electroporation.

After transfection of the host cells, most of the transfectants fail to exhibit the selectable phenotype characteristic of the protein encoded by the selectable gene, but surprisingly a small proportion of the transfectants do exhibit the selectable phenotype, and among those transfectants, the majority are found to express high levels of the desired product encoded by the product gene. Thus, the invention provides an improved method for the selection of recombinant host cells expressing high levels of a desired product, which method is useful with a wide variety of eukaryotic host cells and avoids the problems inherent in existing cell selection technology.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate schematically various DNA constructs encompassed by the instant invention. The large arrows represent the selectable gene and the product gene, the V formed by the dashed lines shows the region of the precursor RNA internal to the 5' splice donor site (SD) and 3' splice acceptor site (SA) that is excised from vectors that contain a functional SD. The transcriptional regulatory region, selectable gene, product gene and transcriptional termination site are depicted in Figure 1A. Figure 1B depicts the DNA constructs of Example 1. The various splice donor sequences are depicted, *i.e.*, wild type ras splice donor sequence (WT ras), mutant ras splice donor sequence (MUTANT ras) and non-functional splice donor sequence (▲GT). The probes used for Northern blot analysis in Example 1 are shown in Figure 1B. Figure 1C depicts the DNA constructs of Example 2 and Figure 1D depicts the DNA construct of Example 3 used for expression of anti-IgE V_H.

Figure 2 depicts schematically the control DNA construct used in Example 1.

Figures 3A-Q depict the nucleotide sequence (SEQ ID NO: 1) of the DHFR/intron-(WT ras SD)-tPA expression vector of Example 1.

Figure 4 is a bar graph which shows the number of colonies that form in selective medium after electroporation of linearized duplicate miniprep DNA's prepared in parallel from the three vectors shown in Figure 1B (*i.e.* with wild type ras splice donor sequence [WT ras], mutant ras splice donor sequence [MUTANT ras] and non-functional splice donor sequence [▲GT]) and from the control vector that has DHFR under control of SV40 promoter and tPA under control of CMV promoter (see Figure 2). Cells were selected in nucleoside free medium and counted with an automated colony counter.

Figures 5A-C are bar graphs depicting expression of tPA from stable pools and clones generated from the vectors shown in Figure 1B. In Figure 5A greater than 100 clones from each vector transfection were mixed, plated in 24 well plates, and assayed by tPA ELISA at "saturation". In Figure 5B, twenty clones chosen at random derived from each of the vectors were assayed by tPA ELISA at "saturation". In Figure 5C, the pools mentioned in Figure 5A (except the ▲GT pool) were exposed to 200nM Mtx to select for DHFR amplification and then pooled and assayed for tPA expression.

Figures 6A-P depict the nucleotide sequence (SEQ ID NO: 2) of the DHFR/intron-(WT ras SD)-TNFr-IgG expression vector of Example 2.

Figures 7A-B are bar graphs depicting expression of TNFr-IgG using dicistronic or control vectors (see Example 2). Vectors containing TNFr-IgG (but otherwise identical to those described for tPA expression in Example 1) were constructed (see Figure 1C), introduced into dp12.CHO cells by electroporation, pooled, and assayed for product expression before (Figure 7A) and after (Figure 7B) being subjected to amplification in 200nM Mtx.

Figure 8 depicts schematically the DNA construct used for expression of the V_L of anti-IgE in Example 3.

Figures 9A-O depict the nucleotide sequence (SEQ ID NO: 3) of the anti-IgE V_H expression vector of Example 3.

Figures 10A-Q depict the nucleotide sequence (SEQ ID NO: 4) of the anti-IgE V_L expression vector of Example 3.

Figure 11 is a bar graph depicting anti-IgE expression in Example 3. Heavy (V_H) and light (V_L) chain expression vectors were constructed, co-electroporated into CHO cells, clones were selected and assayed for antibody expression. Additionally, pools were established and assessed with regard to expression before and after Mtx selection at 200nM and 1μM.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions:

The "DNA construct" disclosed herein comprises a non-naturally occurring DNA molecule which can either be provided as an isolate or integrated in another DNA molecule *e.g.* in an expression vector or the chromosome of an eukaryotic host cell.

The term "selectable gene" as used herein refers to a DNA that encodes a selectable marker necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. Accordingly, a host cell that is transformed with a selectable gene will be capable of growth or survival under certain cell culture conditions wherein a non-transfected host cell is not capable of growth or survival. Typically, a selectable gene will confer resistance to a drug or compensate for a metabolic or catabolic defect in the host cell. Examples of selectable genes are provided in the following table. See also Kaufman, Methods in Enzymology, 185: 537-566 (1990), for a review of these.

TABLE 1

Selectable Genes and their Selection Agents

Selection Agent	Selectable Gene
Methotrexate	Dihydrofolate reductase
Cadmium	Metallothionein
PALA	CAD
Xyl-A-or adenosine and 2'-deoxycoformycin	Adenosine deaminase
Adenine, azaserine, and coformycin	Adenylate deaminase
6-Azauridine, pyrazofuran	UMP Synthetase
Mycophenolic acid	IMP 5'-dehydrogenase

Mycophenolic acid with limiting xanthine	Xanthine-guanine phosphoribosyltransferase
Hypoxanthine, aminopterin, and thymidine (HAT)	Mutant HGPRTase or mutant thymidine kinase
5-Fluorodeoxyuridine	Thymidylate synthetase
Multiple drugs *e.g.* adriamycin, vincristine or colchicine	P-glycoprotein 170
Aphidicolin	Ribonucleotide reductase
Methionine sulfoximine	Glutamine synthetase
β-Aspartyl hydroxamate or Albizziin	Asparagine synthetase
Canavanine	Arginosuccinate synthetase
α-Difluoromethylornithine	Ornithine decarboxylase
Compactin	HMG-CoA reductase
Tunicamycin	*N*-Acetylglucosaminyl transferase
Borrelidin	Threonyl-tRNA synthetase
Ouabain	Na^+K^+-ATPase

The preferred selectable gene is an amplifiable gene. As used herein, the term "amplifiable gene" refers to a gene which is amplified (*i.e.* additional copies of the gene are generated which survive in intrachromosomal or extrachromosomal form) under certain conditions. The amplifiable gene usually encodes an enzyme (*i.e.* an amplifiable marker) which is required for growth of eukaryotic cells under those conditions. For example, the gene may encode DHFR which is amplified when a host cell transformed therewith is grown in Mtx. According to Kaufman, the selectable genes in Table 1 above can also be considered amplifiable genes. An example of a selectable gene which is generally not considered to be an amplifiable gene is the neomycin resistance gene (Cepko *et al.*, *supra*).

As used herein, "selective medium" refers to nutrient solution used for growing eukaryotic cells which have the selectable gene and therefore includes a "selection agent". Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58:44 (1979), Barnes and Sato, Anal. Biochem., 102:255

(1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Patent Re. 30,985; or U.S. Patent No. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The preferred nutrient solution comprises fetal bovine serum.

The term "selection agent" refers to a substance that interferes with the growth or survival of a host cell that is deficient in a particular selectable gene. Examples of selection agents are presented in Table 1 above. The selection agent preferably comprises an "amplifying agent" which is defined for purposes herein as an agent for amplifying copies of the amplifiable gene, such as Mtx if the amplifiable gene is DHFR. See Table 1 for examples of amplifying agents.

As used herein, the term "transcriptional initiation site" refers to the nucleic acid in the DNA construct corresponding to the first nucleic acid incorporated into the primary transcript, *i.e.*, the mRNA precursor, which site is generally provided at, or adjacent to, the 5′ end of the DNA construct.

The term "transcriptional termination site" refers to a sequence of DNA, normally represented at the 3′ end of the DNA construct, that causes RNA polymerase to terminate transcription.

As used herein, "transcriptional regulatory region" refers to a region of the DNA construct that regulates transcription of the selectable gene and the product gene. The transcriptional regulatory region normally refers to a promoter sequence (*i.e.* a region of DNA involved in binding of RNA polymerase to initiate transcription) which can be constitutive or inducible and, optionally, an enhancer (*i.e.* a *cis*-acting DNA element, usually from about 10-300 bp, that acts on a promoter to increase its transcription).

As used herein, "product gene" refers to DNA that encodes a desired protein or polypeptide product. Any product gene that is capable of expression in a host cell may be used, although the methods of the invention are particularly suited for obtaining high-level expression of a product gene that is not also a selectable or amplifiable gene. Accordingly, the protein or polypeptide encoded by a product gene typically will be one that is not necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. For example, product genes suitably encode a peptide, or may encode a polypeptide sequence of

amino acids for which the chain length is sufficient to produce higher levels of tertiary and/or quaternary structure.

Examples of bacterial polypeptides or proteins include, *e.g.*, alkaline phosphatase and β-lactamase. Examples of mammalian polypeptides or proteins include molecules such as renin; a growth hormone, including human growth hormone, and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins such as immunoadhesins and fragments of any of the above-listed polypeptides.

The product gene preferably does not consist of an anti-sense sequence for inhibiting the expression of a gene present in the host. Preferred proteins herein are therapeutic proteins such as TGF-β, TGF-α, PDGF, EGF, FGF, IGF-I, DNase, plasminogen activators such as t-PA, clotting factors such as tissue factor and factor VIII, hormones such as relaxin and insulin, cytokines such as IFN-γ, chimeric proteins such as TNF receptor IgG immunoadhesin (TNFr-IgG) or antibodies such as anti-IgE.

The term "intron" as used herein refers to a nucleotide sequence present within the transcribed region of a gene or within a messenger RNA precursor, which nucleotide sequence is capable of being excised, or spliced, from the messenger RNA precursor by a host cell prior to translation. Introns suitable for use in the present invention are suitably prepared by any of several methods that are well known in the art, such as purification from a naturally occurring nucleic acid or *de novo* synthesis. The introns present in many naturally occurring eukaryotic genes have been identified and characterized. Mount, Nuc. Acids Res., 10:459 (1982). Artificial introns comprising functional splice sites also have been described. Winey *et al.*, Mol. Cell Biol., 9:329 (1989); Gatermann *et al.*, Mol. Cell Biol., 9:1526 (1989). Introns may be obtained from naturally occurring nucleic acids, for example, by digestion of a naturally occurring nucleic acid with a suitable restriction endonuclease, or by PCR cloning using primers complementary to sequences at the 5' and 3' ends of the intron. Alternatively, introns of defined sequence and length may be prepared synthetically using various methods in organic chemistry. Narang *et al.*, Meth. Enzymol., 68:90 (1979); Caruthers *et al.*, Meth. Enzymol., 154:287 (1985); Froehler *et al.*, Nuc. Acids Res., 14:5399 (1986).

As used herein "splice donor site" or "SD" refers to the DNA sequence immediately surrounding the exon-intron boundary at the 5' end of the intron, where the "exon" comprises the nucleic acid 5' to the intron. Many splice donor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. An "efficient splice donor sequence" refers to a nucleic acid sequence encoding a splice donor site wherein the efficiency of splicing of messenger RNA precursors having the splice donor sequence is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. Examples of efficient splice donor sequences include the wild type (WT) ras splice donor sequence and the GAC:GTAAGT sequence of Example 3. Other efficient splice donor sequences can be readily selected using the techniques for measuring the efficiency of splicing disclosed herein.

The terms "PCR" and "polymerase chain reaction" as used herein refer to the *in vitro* amplification method described in US Patent No. 4,683,195 (issued July 28, 1987). In general, the PCR method involves repeated cycles of primer extension synthesis, using two DNA primers capable of hybridizing preferentially to a template nucleic acid comprising the nucleotide sequence to be amplified. The PCR method can be used to clone specific DNA sequences from total genomic DNA, cDNA transcribed from cellular RNA, viral or plasmid DNAs. Wang & Mark, in PCR Protocols, pp.70-75 (Academic Press, 1990); Scharf, in PCR Protocols, pp. 84-98; Kawasaki & Wang, in PCR Technology, pp. 89-97 (Stockton Press, 1989). Reverse transcription-polymerasechain reaction (RT-PCR) can be used to analyze RNA samples containing mixtures of spliced and unspliced mRNA transcripts. Fluorescently tagged primers designed to span the intron are used to

amplify both spliced and unspliced targets. The resultant amplification products are then separated by gel electrophoresis and quantitated by measuring the fluorescent emission of the appropriate band(s). A comparison is made to determine the amount of spliced and unspliced transcripts present in the RNA sample.

One preferred splice donor sequence is a "consensus splice donor sequence". The nucleotide sequences surrounding intron splice sites, which sequences are evolutionarily highly conserved, are referred to as "consensus splice donor sequences". In the mRNAs of higher eukaryotes, the 5' splice site occurs within the consensus sequence AG:GUAAGU (wherein the colon denotes the site of cleavage and ligation). In the mRNAs of yeast, the 5' splice site is bounded by the consensus sequence :GUAUGU. Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986).

The expression "splice acceptor site" or "SA" refers to the sequence immediately surrounding the intron-exon boundary at the 3' end of the intron, where the "exon" comprises the nucleic acid 3' to the intron. Many splice acceptor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. The preferred splice acceptor site is an efficient splice acceptor site which refers to a nucleic acid sequence encoding a splice acceptor site wherein the efficiency of splicing of messenger RNA precursors having the splice acceptor site is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. The splice acceptor site may comprise a consensus sequence. In the mRNAs of higher eukaryotes, the 3' splice acceptor site occurs within the consensus sequence $(U/C)_{11}$NCAG:G. In the mRNAs of yeast, the 3' acceptor splice site is bounded by the consensus sequence (C/U)AG:. Padgett, *et al.*, *supra*.

As used herein "culturing for sufficient time to allow amplification to occur" refers to the act of physically culturing the eukaryotic host cells which have been transformed with the DNA construct in cell culture media containing the amplifying agent, until the copy number of the amplifiable gene (and preferably also the copy number of the product gene) in the host cells has increased relative to the transformed cells prior to this culturing.

The term "expression" as used herein refers to transcription or translation occurring within a host cell. The level of expression of a product gene in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook,

et al., Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

Modes for Carrying Out the Invention

Methods and compositions are provided for enhancing the stability and/or copy number of a transcribed sequence in order to allow for elevated levels of a RNA sequence of interest. In general, the methods of the present invention involve transfecting a eukaryotic host cell with an expression vector comprising both a product gene encoding a desired polypeptide and a selectable gene (preferably an amplifiable gene).

Selectable genes and product genes may be obtained from genomic DNA, cDNA transcribed from cellular RNA, or by *in vitro* synthesis. For example, libraries are screened with probes (such as antibodies or oligonucleotides of about 20-80 bases) designed to identify the selectable gene or the product gene (or the protein(s) encoded thereby). Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the selectable gene or product gene is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues known to contain the selectable gene or product gene. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide generally is labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P- labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Sometimes, the DNA encoding the selectable gene and product gene is preceded by DNA encoding a signal sequence having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the expression vector, or it may be a part of the selectable gene or product gene that is inserted into the expression vector. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence

of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. The DNA for such precursor region is ligated in reading frame to the selectable gene or product gene.

As shown in Figure 1A, the selectable gene is generally provided at the 5' end of the DNA construct and this selectable gene is followed by the product gene. Therefore, the full length (non-spiced) message will contain DHFR as the first open reading frame and will therefore generate DHFR protein to allow selection of stable transfectants. The full length message is not expected to generate appreciable amounts of the protein of interest as the second AUG in a dicistronic message is an inefficient initiator of translation in mammalian cells (Kozak, J. Cell Biol., 115: 887-903 [1991]).

The selectable gene is positioned within an intron. Introns are noncoding nucleotide sequences, normally present within many eukaryotic genes, which are removed from newly transcribed mRNA precursors in a multiple-step process collectively referred to as splicing.

A single mechanism is thought to be responsible for the splicing of mRNA precursors in mammalian, plant, and yeast cells. In general, the process of splicing requires that the 5' and 3' ends of the intron be correctly cleaved and the resulting ends of the mRNA be accurately joined, such that a mature mRNA having the proper reading frame for protein synthesis is produced. Analysis of a variety of naturally occurring and synthetically constructed mutant genes has shown that nucleotide changes at many of the positions within the consensus sequences at the 5' and 3' splice sites have the effect of reducing or abolishing the synthesis of mature mRNA. Sharp, Science, 235:766 (1987); Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986); Green, Ann. Rev. Genet., 20:671 (1986). Mutational studies also have shown that RNA secondary structures involving splicing sites can affect the efficiency of splicing. Solnick, Cell, 43:667 (1985); Konarska, *et al.*, Cell, 42:165 (1985).

The length of the intron may also affect the efficiency of splicing. By making deletion mutations of different sizes within the large intron of the rabbit beta-globin gene, Wieringa, *et al.* determined that the minimum intron length necessary for correct splicing is about 69 nucleotides. Cell, 37:915 (1984). Similar studies of the intron of the adenovirus E1A region have shown that an intron length of about 78 nucleotides allows correct splicing to occur, but at reduced efficiency. Increasing the length of the intron to 91 nucleotides restores normal splicing efficiency, whereas truncating the intron to 63 nucleotides abolishes correct splicing. Ulfendahl, *et al.*, Nuc. Acids Res., 13:6299 (1985).

To be useful in the invention, the intron must have a length such that splicing of the intron from the mRNA is efficient. The preparation of introns of differing lengths is a routine matter, involving methods well known in the art, such as *de novo* synthesis or *in vitro* deletion

mutagenesis of an existing intron. Typically, the intron will have a length of at least about 150 nucleotides, since introns which are shorter than this tend to be spliced less efficiently. The upper limit for the length of the intron can be up to 30 kB or more. However, as a general proposition, the intron is generally less than about 10 kB in length.

The intron is modified to contain the selectable gene not normally present within the intron using any of the various known methods for modifying a nucleic acid *in vitro*. Typically, a selectable gene will be introduced into an intron by first cleaving the intron with a restriction endonuclease, and then covalently joining the resulting restriction fragments to the selectable gene in the correct orientation for host cell expression, for example by ligation with a DNA ligase enzyme.

The DNA construct is dicistronic, *i.e.* the selectable gene and product gene are both under the transcriptional control of a single transcriptional regulatory region. As mentioned above, the transcriptional regulatory region comprises a promoter. Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7:149 [1968]; and Holland, Biochemistry, 17:4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide.

Product gene transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the product gene, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus (CMV) is conveniently obtained as a *Hin*dIII E restriction fragment. Greenaway *et al.*, Gene, 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

Preferably the transcriptional regulatory region in higher eukaryotes comprises an enhancer sequence. Enhancers are relatively orientation and position independent having been found 5' (Lainins *et al.*, Proc. Natl. Acad. Sci. USA, 78:993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3:1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33:729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4:1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer (CMV), the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the product gene, but is preferably located at a site 5' from the promoter.

The DNA construct has a transcriptional initiation site following the transcriptional regulatory region and a transcriptional termination region following the product gene (see Figure 1A). These sequences are provided in the DNA construct using techniques which are well known in the art.

The DNA construct normally forms part of an expression vector which may have other components such as an origin of replication (*i.e.*, a nucleic acid sequence that enables the vector to replicate in one or more selected host cells) and, if desired, one or more additional selectable gene(s). Construction of suitable vectors containing the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA

fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Generally, in cloning vectors the origin of replication is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known. The 2μ plasmid origin of replication is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

For analysis to confirm correct sequences in plasmids constructed, plasmids from the transformants are prepared, analyzed by restriction, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9:309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65:499 (1980).

The expression vector having the DNA construct prepared as discussed above is transformed into a eukaryotic host cell. Suitable host cells for cloning or expressing the vectors herein are yeast or higher eukaryote cells.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing the product gene. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, 290:140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112:284-289 (1983); Tilburn *et al.*, Gene, 26:205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81:1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4:475-479 (1985)].

Suitable host cells for the expression of the product gene are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda*

(caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosphila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6:47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315:592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the product gene. During incubation of the plant cell culture with *A. tumefaciens*, the product gene is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the product gene. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 [1980]); dp12.CHO cells (EP 307,247 published 15 March 1989); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be used. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

In the preferred embodiment the DNA is introduced into the host cells using electroporation. See Andreason, J. Tiss. Cult. Meth., 15:56-62 (1993), for a review of electroporation techniques useful for practicing the instantly claimed invention. It was discovered that electroporation techniques for introducing the DNA construct into the host cells were preferable over calcium phosphate precipitation techniques insofar as the latter could cause the DNA to break up and forming concantemers.

The mammalian host cells used to express the product gene herein may be cultured in a variety of media as discussed in the definitions section above. The media contains the selection agent used for selecting transformed host cells which have taken up the DNA construct (either as an intra- or extra-chromosomal element). To achieve selection of the transformed eukaryotic cells, the host cells may be grown in cell culture plates and individual colonies expressing the selectable gene (and thus the product gene) can be isolated and grown in growth medium until the nutrients are depleted. The host cells are then analyzed for transcription and/or transformation as discussed below. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescens, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the

formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75:734-738 (1980).

In the preferred embodiment, the mRNA is analyzed by quantitative PCR (to determine the efficiency of splicing) and protein expression is measured using ELISA as described in Example 1 herein.

The product of interest preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal. When the product gene is expressed in a recombinant cell other than one of human origin, the product of interest is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the product of interest from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the product of interest. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The product of interest thereafter is purified from contaminant soluble proteins and polypeptides, for example, by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel electrophoresis using, for example, Sephadex G-75; chromatography on plasminogen columns to bind the product of interest and protein A Sepharose columns to remove contaminants such as IgG.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated by reference.

EXAMPLE 1

tPA production using the dicistronic expression vectors

It was sought to increase the level of homogeneity with regard to expression levels of stable clones by expressing a selectable marker (such as DHFR) and the protein of interest from a single promoter. These vectors divert most of the transcript to product expression while linking it at a fixed ratio to DHFR expression via differential splicing.

Vectors were constructed which were derived from the vector pRK (Suva *et al.*, Science, 237:893-896 [1987]) which contains an intron between the cytomegalovirus immediate early promoter (CMV) and the cDNA that encodes

the polypeptide of interest. The intron of pRK is 139 nucleotides in length, has a splice donor site derived from cytomegalovirus immediate early gene (CMVIE), and a splice acceptor site from an IgG heavy chain variable region (V_H) gene (Eaton *et al.*, Biochem., 25:8343 [1986]).

DHFR/intron vectors were constructed by inserting an EcoRV linker into the BSTX1 site present in the intron of pRK7. An 830 base-pair fragment containing a mouse DHFR coding fragment was inserted to obtain DHFR intron expression vectors which differ only in the sequence that comprises the splice donor site. Those sequences were altered by overlapping PCR mutagenesis to obtain sequences that match splice donor sites found between exons 3 and 4 of normal and mutant Ras genes. PCR was also used to destroy the splice donor site.

A mouse DHFR cDNA fragment (Simonsen *et al.*, Proc. Natl. Acad. Sci. USA, 80:2495-2499 [1983]) was inserted into the intron of this vector 59 nucleotides downstream of the splice donor site. The splice donor site of this vector was altered by mutagenesis to change the ratio of spliced to non-spliced message in transfected cells. It has previously been shown that a single nucleotide change (G to A) converted a relatively efficient splice donor site found in the normal ras gene into an inefficient splice site (Cohen *et al.*, Nature, 334:119-124 [1988]). This effect has been demonstrated in the context of the ras gene and confirmed when these sequences were transferred to human growth hormone constructs (Cohen *et al.*, Cell, 58:461-472 [1989]). Additionally, a non functional 5' splice site (GT to CA) was constructed as a control (ΔGT). A polylinker was inserted 35 nucleotides downstream of the 3' splice site to accept the cDNA of interest. A vector containing tPA (Pennica *et al.*, Nature, 301:214-221 [1983]) was linearized downstream of the polyadenylation site before it was introduced into CHO cells (Potter *et al.*, Proc. Natl. Acad. Sci. USA, 81:7161 [1984]).

Plasmid DNA's that contained DHFR/intron, tPA and (a) wild type ras (WT ras), *i.e.* Figure 3 (SEQ ID NO: 1), (b) mutant ras, or (c) non-functional splice donor site (ΔGT) were introduced into CHO DHFR minus cells by electroporation. The intron vectors were each linearized downstream of the polyadenylation site by restriction endonuclease treatment. The control vector was linearized downstream of the second polyadenylation site. The DNA's were ethanol precipitated after phenol/chloroform extraction and were resuspended in 20μl 1/10 Tris EDTA. Then, 10μg of DNA was incubated with 10^7 CHO.dp12 cells (EP 307,247 published 15 March 1989) in 1 ml of PBS on ice for 10 min. before electroporation at 400 volts and 330μf using a BRL Cell Porator.

Cells were returned to ice for 10 min. before being plated into non-selective medium. After 24 hours cells were fed nucleoside-free medium to select for stable DHFR+ clones which were pooled. The pooled DHFR+ clones were lysed and mRNA's were prepared.

To prepare the mRNA, RNA was extracted from 5 x 10^7 cells which were grown from pools of more than 200 clones derived from the stable

transfection of the three vectors, the essential construction of which is shown in Figure 1B and from non-transfected CHO cells. RNA was purified over oligo-DT cellulase (Collaborative Biomedical Products). 10μg of mRNA was then subjected to Northern blotting which involved running the mRNA on a 1.2% agarose, 6.6% formaldehyde gel, and transferring it to a nylon filter (Stratagene Duralon-UV membrane), prehybridized, probed and washed according to the manufacturer's instructions.

The filter was probed sequentially using probes (shown in Figure 1B) that would detect (a) the full length message, (b) both full length and spliced message, or (c) beta actin. Probing with the long probe showed that the vector that contains the efficient splice donor site (*i.e.* WT ras) generates predominately a mRNA of the size predicted for the spliced product while the other two vectors gave rise primarily to a mRNA that corresponds in size to non-spliced message. The DHFR probe detected only full length message and demonstrated that the WT ras splice donor derived vector generates very little full length message with which to confer a DHFR positive phenotype.

Figure 4 shows the number of DHFR positive colonies obtained after duplicate electroporations with the three intron vectors described above and from a conventional vector that has a CMV promoter driving tPA and a SV40 promoter driving DHFR (see Figure 2). The increase in colony number parallels the increase in full length message that accumulates with the modification of the splice donor sites. The conventional vector efficiently generates colonies and does not vary significantly from the ΔGT construct.

The level of tPA expression was determined by seeding cells in 1 ml of F12:DMEM (50:50, with 5% FBS) in 24 well dishes to near confluency. Growth of the cells continued until the media was exhausted. Media was then assayed by ELISA for tPA production. Briefly, anti-tPA antibody was coated onto the wells of an ELISA microtiter plate, media samples were added to the wells followed by washing. Binding of the antigen (tPA) was then quantified using horse radish peroxidase (HRPO) labelled anti-tPA antibody.

Figure 5A depicts the titers of secreted tPA protein after pooling the clones of each group shown in Figure 4. While the number of colonies increased with a weakening of splice donor function, the inverse was seen with respect to tPA expression. The expression levels are consistent with the RNA products that are observed; as more of the dicistronic message is spliced an increased amount of message will contain tPA as the first open reading frame resulting in increased tPA expression. A mutation of GT to CA in the splice donor site results in an abundance of DHFR positive colonies which express undetectable levels of tPA, possibly resulting from inefficient utilization of the second AUG. Importantly, Figure 5A also shows that expression levels obtained from one of the dicistronic vectors (with WT ras SD) was about threefold higher than that obtained with the control vector containing a CMV promoter/enhancer driving tPA, SV40

promoter/enhancer controlling DHFR and SV40 polyadenylation signals controlling the expression of tPA and DHFR.

Additionally, the homogeneity of expression in the pools was investigated. Figure 5B shows that all 20 clones generated by the WT ras splice donor site derived dicistronic vectors express detectable levels of tPA while only 4 of 20 clones generated by the control vector express tPA. None of the clones transfected with the non-splicing (ΔGT) vector expressed tPA levels detectable by ELISA. This finding is consistent with previous observations that relatively few clones generated by conventional vectors make useful levels of protein.

Expression of tPA was increased following methotrexate amplification of pools. Figure 5C shows that 2 of the dicistronic vector derived pools (*i.e.* with WT ras and MUTANT ras SD sites) increased in expression markedly (8.4 and 7.7 fold), while the pool generated by the conventional vector increased only slightly (2.8 fold) when each was subjected to 200 nM Mtx. An overall increase of 9 fold was obtained using the best dicistronic (WT ras SD) versus the conventional vector following amplification. Growth of the highest expressing amplified pool in nutrient rich production medium yielded titers of 4.2 μg/ml tPA.

It was shown that manipulation of the splice donor sequence alters the ratio of spliced to full length message and the number of colonies that form in selective medium. It was also shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Surprisingly, it was possible to isolate high expressors which had the efficient WT ras splice donor site by selection for DHFR$^-$ cells despite the efficiency with which the DHFR gene was spliced from the RNA precursors formed in these cells.

EXAMPLE 2

TNFr-IgG production using the dicistronic expression vectors

To prove the general applicability of this approach, a second product was evaluated in the dicistronic vector system containing, as the DNA of interest, an immunoadhesin (TNFr-IgG) capable of binding tumor necrosis factor (TNF) (Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA, 88:10535-10539 [1991]). The experiments described in Example 1 above were essentially repeated except that the product gene encoded the immunoadhesin TNFr-IgG. Plasmid DNA's that contained a TNFr-IgG cDNA and (a) WT ras, *i.e.* Figure 6 (SEQ ID NO: 2), (b) mutant ras or (c) nonfunctional splice donor site (ΔGT) were introduced into the dp12.CHO cells as discussed for Example 1. See Figure 1C for an illustration of the DNA constructs.

It was discovered that the number of DHFR positive colonies generated by three of these vectors was similar to that seen with the tPA constructs. Expression of TNFr-IgG also paralleled that seen with the tPA constructs (Figure 7A). Amplification of pools from two of the constructs showed a marked increase in expression of immunoadhesin (9.6 and 6.8 fold) (Figure

7B). The best of these amplified pools expressed 9.5 μg/ml when grown in nutrient rich production medium.

Thus, it was again shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Furthermore, contrary to expectations, it was discovered that isolation of high product expressing host DHFR$^-$ cells was possible using an efficient splice donor site (*i.e.* the WT ras splice donor site).

EXAMPLE 3

Antibody production using a dicistronic expression vector

The usefulness of this system for antibody expression was evaluated by testing production of an antibody directed against IgE (Presta *et al.*, Journal of Immunology, 151:2623-2632 [1993]). Further, the flexibility of the system with regard to transcription initiation was tested by replacing the CMV promoter/enhancer present in the previous vectors with the promoter/ enhancer derived from the early region of SV40 virus (Griffin, B., Structure and Genomic Organization of SV40 and Polyoma Virus, In J. Tooze [Ed] DNA Tumor Viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The heavy chain of the antibody was inserted downstream of DHFR as described in the earlier tPA and TNFr-IgG constructs. Additionally, a new splice donor site sequence (GAC:GTAAGT) was engineered into the vector which matches the consensus splice donor site more closely than did the splice donor sites present in the vectors tested in Examples 1 and 2. The resultant expression vector is shown in Figures 1D and 9.

It was discovered that this vector produced fewer colonies than the vectors previously tested, and produced predominantly a spliced RNA product. A second vector was constructed to have the light chain of the antibody under control of the SV40 promoter/enhancer and poly-A and the hygromycin B resistance gene under control of the CMV promoter/enhancer and SV40 poly-A. These vectors were linearized at unique HpaI sites downstream of the poly-A signal, mixed at a ratio of light chain vector to heavy chain vector of 10:3 and electroporated into CHO cells using an optimized protocol (as discussed in Examples 1 and 2).

Figure 11 shows the levels of antibody expressed by clones and pools after selection in hygromycin B followed by selection for DHFR expression. All 20 of the clones analyzed expressed high levels of antibody when grown in rich medium and varied from one another by only a factor of four. A pool of antibody producing clones was generated and assayed shortly after it was established. That pool was grown continuously for 6 weeks without a significant decrease in productivity demonstrating that its stability was sufficient to generate gram quantities of protein from its large scale culture.

The pool was subjected to methotrexate amplification at 200nM and 1μM and achieved a greater than 2 fold increase in antibody titer. The 1μM Mtx resistant pool achieved a titer of 41 mg/L when grown under optimal conditions in suspension culture.

The structure of the expressed antibody was examined. Proteins expressed by the 200nM methotrexate resistant pool and by a well characterized expression clone generated by conventional vectors (Presta *et al.* [1993], *supra*) were metabolically labeled with S^{35} cysteine and methionine. In particular, confluent 35mm plates of cells were metabolically labeled with 50μCi each S-35 methionine and S-35 cysteine (Amersham) in serum free cysteine and methionine free F12:DMEM. After one hour, nutrient rich production media was added and labeled proteins were allowed to "chase" into the medium for six more hours. Proteins were run on a 12% SDS/PAGE gel (NOVEX) non-reduced or following reduction with B-mercaptoethanol. Dried gels were exposed to film for 16 hours. CHO control cells were also labeled.

The majority of the antibody protein is secreted with a molecular weight of about 155 kilodaltons, consistent with a properly disulfide-linked antibody molecule with 2 light and 2 heavy chains. Upon reduction the molecular weight shifts to 2 approximately equally abundant proteins of 22.5 and 55 kilodaltons. The protein generated from the pool is indistinguishable from the antibody produced by the well characterized expression clone, with no apparent increase of free heavy or light chain expressed by the pool.

CONCLUSION

The efficient expression system described herein utilizes vectors consisting of promoter/enhancer elements followed by an intron containing the selectable marker coding sequence, followed by the cDNA of interest and a polyadenylation signal.

Several splice donor site sequences were tested for their effect on colony number and expression of the cDNA of interest. A non-functional splice donor site, splice donor sites found in an intron between exons 3 and 4 of mutant (mutant ras) and normal (WT ras) forms of the Harvey Ras gene and another efficient SD site (see Example 3) were used. The vectors were designed to direct expression of dicistronic primary transcripts. Within a transfected cell some of the transcripts remain full length while the remainder are spliced to excise the DHFR coding sequence. When the splice donor site is weakened or destroyed an increase in colony number is observed.

Expression levels show the inverse pattern, with the most efficient splice donor sites generating the highest levels of tPA, TNFr immunoadhesin or anti-IgE V_H.

The homogeneity of expression of clones generated by the ras splice donor site intron DHFR vectors was compared to clones generated from a conventional vector with a separate promoter/enhancer and polyadenylation signal for each DHFR and tPA. The DHFR intron vector gives rise to colonies that are much more homogeneous with regard to expression than those generated by the conventional vector. Non-expressing clones derived from the conventional vector may be the result of breaks in the tPA or

TNFr-IgG domain of the plasmid during integration into the genome or the result of methylation of promoter elements (Busslinger *et al.*, Cell, 34:197-206 [1983]; Watt *et al.*, Genes and Development, 2:1136-1143 [1988]) driving tPA or TNFr-IgG expression. Promoter silencing by methylation or breaks in the DHFR-intron vectors would very likely render them incapable of conferring a DHFR positive phenotype.

It was found that pools generated by the DHFR-intron vectors could be amplified in methotrexate and would increase in expression by a factor of 8.4 (tPA), or 9.8 (TNFr-IgG). Pools from conventional vectors increased by only 2.8 and 3.0 fold for tPA and TNFr-IgG when amplified similarly. Amplified pools resulted in 9 fold higher tPA levels and 15 fold higher TNFr-IgG levels when compared to the conventional vector amplified pools.

Without being limited to any theory, the increase in expression of methotrexate resistant pools derived from the dicistronic vectors is likely due to the transcriptional linkage of DHFR and the product; when cells are selected for increased DHFR expression they consistently over-express product. Conventional approaches lack selectable marker and cDNA expression linkage and therefore methotrexate amplification often generates DHFR overexpression without the concomitant increase in product expression.

A further increase of 4 and 6.3 fold in expression were obtained when amplified tPA and TNFr-IgG pools were transferred from the media used for the selections and amplifications to a nutrient rich production medium.

In Example 3, the expression vector had a splice donor site that more closely matches the consensus splice donor sequence and had the heavy chain of a humanized anti-IgE antibody inserted downstream. This vector was linearized and co-electroporated with a second linearized vector that expresses the hygromycin resistance gene and the light chain of the antibody each under the control of its own promoter/enhancer and poly-A signals. An excess of light chain expression vector over the heavy chain dicistronic expression vector was used to bias in favor of light chain expression. Clones and a pool were generated after hygromycin B and DHFR selections. The clones were found to express relatively consistent, high levels of antibody, as did the pool. The 1μM pool achieved a titer of 41mg/L when grown under optimal conditions in suspension culture.

The anti-IgE antibody was assessed by metabolic labeling followed by SDS/PAGE under reducing and non reducing conditions and found to be indistinguishable from the protein expressed by a highly characterized clonal cell line. Of particular importance is the finding that no free light chain is observed in the pool relative to the clone.

A stable expression system for CHO cells has been developed that produces high levels of recombinant proteins rapidly and with less effort than that required by other expression systems. The vector system generates stable clones that express consistently high levels thereby reducing the number of clones that must be screened to obtain a highly productive clonal line. Alternatively, pools have been used to conveniently generate moderate to high levels of protein. This approach

may be particularly useful when a number of related proteins are to be expressed and compared.

Without being limited to this theory, it is possible the vectors that have very efficient splice donor sites generate very productive clones because so little transcript remains non spliced that only integration events that lead to the generation of high levels of RNA produce enough DHFR protein to give rise to colonies in selective medium. The high level of spliced message from such clones is then translated into abundant amounts of the protein of interest. Pools of clones made concurrently by introducing conventional vectors expressed lower levels of protein, and were unstable with regard to long term expression, and expression could not be appreciably increased when the cells were subjected to methotrexate amplification.

The system developed herein is versatile in that it allows high levels of single and multiple subunit polypeptides to be rapidly generated from clones or pools of stable transfectants. This expression system combines the advantages of transient expression systems (rapid and labor non intensive generation of research amounts of protein) with the concurrent development of highly productive stable production cell lines.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENENTECH, INC.

(ii) TITLE OF INVENTION: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/286740
(B) FILING DATE: 05-AUG-1994

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7360 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA 750

GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT 800

GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA 850

CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC 900

AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA 950

CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT 1000

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT 1600

AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG 1650

TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC 1700

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GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC 1850

AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC 1900

GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG 1950

CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT 2000

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CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG 2650

GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA 2700

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GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT 2800

GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT 2850

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TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG 3450

GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT 3500

GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA 3550

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GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA 3650

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TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA 3750

TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA 3800

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TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC 5500

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GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT 5600

ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC 5650

ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC 5700

ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC 5750

GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC 5800

TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT 5850

GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC 5900

TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG 5950

AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC 6000

TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT 6050

CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT 6100

ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT 6150

GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA 6200

CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA 6250

TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT 6300

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TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA 6400

AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT 6450

CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT 6500

CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC 6550

CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT 6600

GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA 6650

TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT 6700

TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA 6750

GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG 6800

CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG 6850

CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT 6900

CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG 6950

CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA 7000

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TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA 7250

TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG 7300

GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT 7350

TACGAATTAA 7360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6889 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

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AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC 1600

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TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG 2900

CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG 2950

CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC 3000

AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC 3050

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GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT 3150

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TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA 3250

ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC 3300

GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC 3350

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ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT 4250

TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG 4300

GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT 4350

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TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT 4900

TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT 4950

GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA 5000

CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA 5050

TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC 5100

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CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT 6850

TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA 6889

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6557 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG 50

GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA 100

GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG 150

TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA 200

GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC 250

CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT 300

TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG 350

AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG 400

CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA 450

GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT 500

TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA 550

AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC 600

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TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA 700

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GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA 800

ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA 850

GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT 900

GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT 950

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TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC 2000

CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT 2050

GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC 2100

TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT 2150

CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA 2200

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CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG 2500

ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG 2550

ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA 2600

GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT 2650

CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 2700

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AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG 5500

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TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC 5750

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AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA 5950

AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG 6000

CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT 6050

GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA 6100

TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA 6150

CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT 6200

TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT 6250

GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC 6300

AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG 6350

CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG 6400

AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA 6450

GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA 6500

TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC 6550

GAATTAA 6557

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7305 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750

GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA 800

CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850

CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900

ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG 950

TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT 1000

GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG 1050

GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA 1100

GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA 1150

AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC 1200

GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC 1250

GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA 1300

TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC 1350

AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG 1400

TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA 1450

GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC 1500

ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC 1550

CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA 1600

ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG 1650

AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG 1700

CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG 1750

CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG 1800

ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC 1850

CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA 1900

TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA 1950

GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC 2000

CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA 2050

CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG 2100

TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT 2150

TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA 2200

GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG 2250

GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC 2300

ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA 2350

GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC 2400

GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT 2450

CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC 2500

GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG 2550

CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG 2600

AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT 2650

ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA 2700

ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC 2750

CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC 2800

TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT 2850

TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA 2900

GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT 2950

GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG 3000

TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC 3050

GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA 3100

CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT 3150

TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC 3200

CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC 3250

CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG 3300

CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA 3350

CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA 3400

AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC 3450

TACGAGAAAC ACAAAGTCTA CGCCTGCGAA GTCACCCATC AGGGCCTGAG 3500

CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAA GCTTCGATGG 3550

CCGCCATGGC CCAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG 3600

CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA 3650

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CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG 3750

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GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG 4650

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ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA 4750

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CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT 4950

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AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT 5250

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ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT 5600

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CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG 5850

AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG 5900

GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT 5950

TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG 6000

CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG 6050

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ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG 6200

GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT 6250

TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT 6300

GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA 6350

CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT 6400

TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC 6450

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TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG 6600

CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG 6650

CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG 6700

CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA 6750

GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG 6800

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TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG 6900

CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC 6950

TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA 7000

GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG 7050

TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG 7100

CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA 7150

AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG 7200

CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT 7250

GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA 7300

ATTAA 7305

CLAIMS

1. A DNA construct comprising a transcriptional initiation site, a transcriptional termination site, a selectable gene, a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene being positioned within an intron having a splice donor site 5' of the intron, which splice donor site regulates expression of the product gene using the transcriptional regulatory region.

2. The DNA construct of claim 1 wherein the splice donor site comprises an efficient splice donor sequence.

3. The DNA construct of claim 2 wherein the splice donor site comprises a consensus splice donor sequence.

4. The DNA construct of claim 2 wherein the splice donor site comprises the sequence GACGTAAGT.

5. The DNA construct of claim 1 wherein the selectable gene is an amplifiable gene.

6. The DNA construct of claim 5 wherein the amplifiable gene is DHFR.

7. The DNA construct of claim 1 wherein the transcriptional regulatory region comprises a promoter and an enhancer.

8. A vector comprising the DNA construct of claim 1.

9. The vector of claim 8 wherein the selectable gene of the DNA construct is an amplifiable gene.

10. The vector of claim 8 that is capable of replication in a eukaryotic host.

11. A eukaryotic host cell comprising the vector of claim 10.

12. A eukaryotic host cell comprising the DNA construct of claim 5.

13. The host cell of claim 11 wherein the vector is introduced into the host cell by electroporation.

14. A eukaryotic host cell comprising the DNA construct of claim 1 integrated into a chromosome of the host cell.

15. The host cell of claim 14 that is a mammalian cell.

16. A method for producing a product of interest comprising culturing the host cell of claim 11 so as to express the product gene and recovering the product from the host cell culture.

17. The method of claim 16 further comprising recovering the product from the culture medium.

18. The method of claim 16 wherein the selectable gene is an amplifiable gene and the splice donor site comprises an efficient splice donor sequence.

19. A method for producing a product of interest comprising culturing the host cell of claim 12 so as to express the product gene in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, and recovering the product.

20. A method for producing eukaryotic cells having multiple copies of a product gene comprising transforming eukaryotic cells with the DNA construct of claim 5, growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene.

21. The method of claim 20 further comprising recovering from the selected cells the product of interest.

FIG. 1A

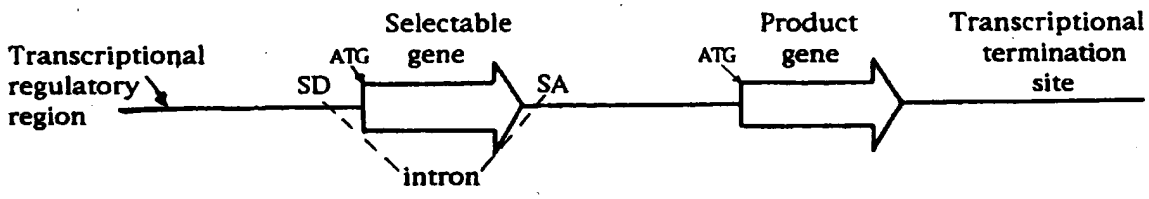
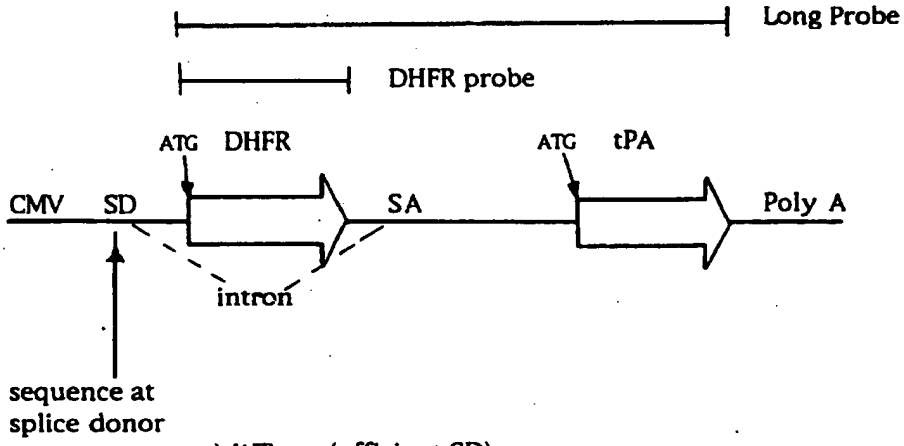
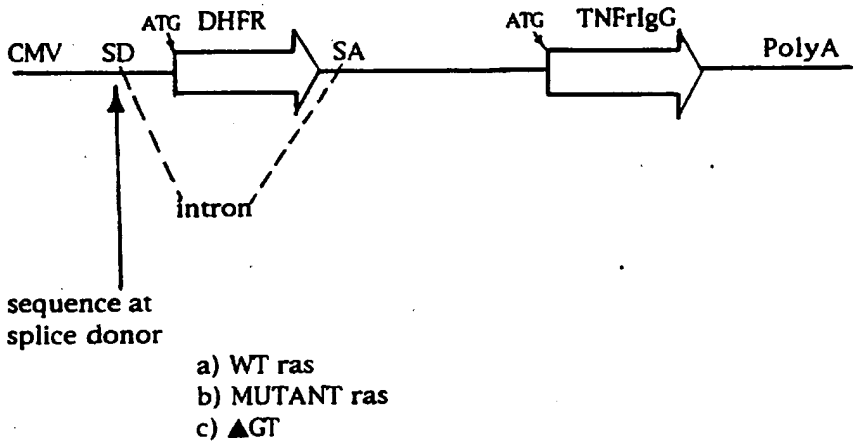


FIG. 1B



a) WT ras (efficient SD)
b) MUTANT ras (less efficient SD)
c) ▲GT (inefficient SD)

FIG. 1C



a) WT ras
b) MUTANT ras
c) ▲GT

FIG. 1D

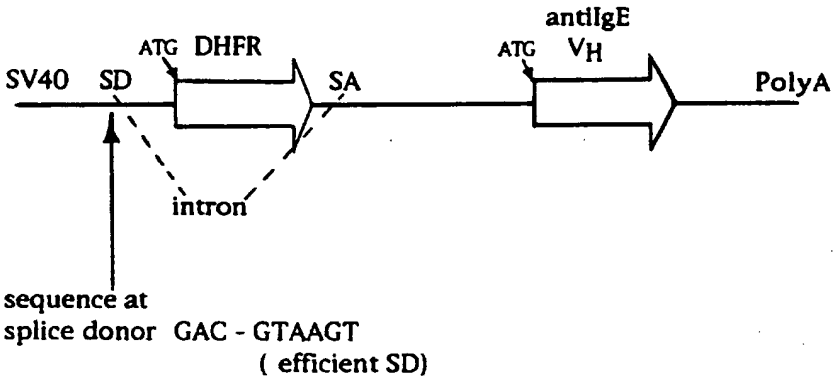

antiIgE
ATG DHFR
ATG V_H
SV40 SD
SA
PolyA
intron
sequence at
splice donor GAC - GTAAGT
(efficient SD)

FIG. 2

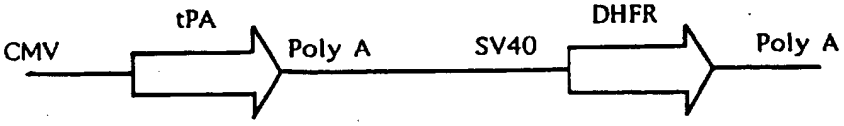

tPA
DHFR
CMV
Poly A
SV40
Poly A

FIG. 3A

```
      aluI
     sstI
     sacI
     hgiJII
     hgiAI/aspHI
     ecl136II
     bsp1286
     bsiHKAI
     bmyI                          rmaI     tru9I                                                  thaI
     banII                         maeI     mseI                                                   fnuDII/mvnI
    taqI                          speI     aseI/asnI/vspI                              bslI        bstUI
                                                                                                   bsh1236I
                                                                                                  aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                 scrFI
                 mvaI
                 ecoRII
                 dsaV
               aciI
             bglI bstNI                               maeII
            sau96I                                   hinlI/acyI
            haeIII/palI     aciI                     ahaII/bsaHI
            asuI  apyI(dcm+)             aciI        aatII          maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

      maeII                                                                                       maeII
     hinlI/acyI                                                                                  hinlI/acyI
     ahaII/bsaHI                                  rsaI                       rsaI                ahaII/bsaHI
     aatII                           bglI         csp6I         ndeI         csp6I               aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

           scrFI
           mvaI
           ecoRII
        aciI                                                                                        nlaIII
      bglI dsaV                                                                               styI
      sau96I bstNI        rsaI                                            maeII                   ncoI
      haeIII/palI         csp6I                                 rsaI     snaBI                    dsaI hphI  aciI
      asuI   apyI(dcm+)  bsrI  nlaIII                           csp6I    bsaAI                    bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 3B

```
                                                                                       maeII
                                                                                       hinlI/acyI               nlaIV
      rsaI                                       pleI                                  ahaII/bsaHI              hgiCI
      csp6I                          aciI        hinfI                 bsmAI           aatII                    banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                          aluI
                                                                                                         sstI
                                                                                                         sacI
                                                                                                         hgiJII
                                                                                                         hgiAI/aspHI
                                                                                                         ecl136II
                                                                                                         bsp1286
                                                                                                         bsiHKAI
                                                                                rsaI                     bmyI
                             maeIII     aciI        hgaI        aciI             csp6I      mnlI          banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                haeIII/palI
                                                                                               mcrI
                                                                                               eagI/xmaIII/eclXI
                                                                                               eaeI
                                                                                               cfrI
                                                                                              fnu4HI
                                                                                              aciI
                                                                                             thaI
                        esp3I                                                                fnuDII/mvnI
                    scrFI                                          sau96I                sacII/sstII
                    mvaI bsmAI                                          avaII                 nspBII
                    ecoRII                                         asuI                  kspI  scrFI
                    dsaV                                          nlaIV                  dsaI  nciI
                    bstNI  hinlI/acyI                                 scrFI               bglI bslI  mspI
                    apyI[dcm+]                                        nciI            sau3AI mnlI bstUI
                sau3AI gsuI/bpmI                                      mspI            mboI/ndeII[dam-] hpaII
                mboI/ndeII[dam-]                               mboII  hpaII           dpnI[dam+] bsaJI dsaV
                dpnI[dam+]  hgaI  fokI                         bpuAI  dsaV            dpnII[dam-] bsh1236I
                dpnII[dam-] ahaII/bsaHI              mnlI      bbsI   cauII           alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 3C

```
            tfiI
         aciI
        thaI hinfI
        fnuDII/mvnI
        bstUI                                          aciI                                            fnu4HI
        bsh1236I                                       rsaI                                            bbvI
                                                       csp6I   scfI          mnlI                      nspBII            taqI
701  TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                                 thaI
                                                                                                 fnuDII/mvnI
                                                                                                 bstUI
                                                                                                 bsh1236I
                                                                                               mluI
                                                                          bsrBI                aflIII       rsaI
                pflMI                            bsmAI                    aciI               xmnI           csp6I
     sfaNI      bslI                             bsaI                   mnlI    ddeI         asp700        scaI
801  GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                            scrFI
                                                            mvaI
                                                            ecoRII
        eco57I                                              dsaV
       mboII                 tfiI                           bstNI                      tfiI      tru9I          tru9I
      earI/ksp632I           hinfI                          apyI[dcm+]           mboII   taqI    mseI           mseI
     mnlI                   alwNI      hphI               sexAI             ddeI     hinfI      ahaIII/draI    aseI/asnI/vspI
901  AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                               aluI
                                               sstI
                                               sacI
                                               hgiJII
                                               hgiAI/aspHI
                                               ecl136II
                                               bsp1286
                                               bsiHKAI
                                               bmyI                                            tru9I
                                               banII                            sfaNI          mseI            mspI
      ddeI                          bslI    mnlI                  bstXI       fokI           aflII/bfrI        hpaII
                                                                                                             bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 3D

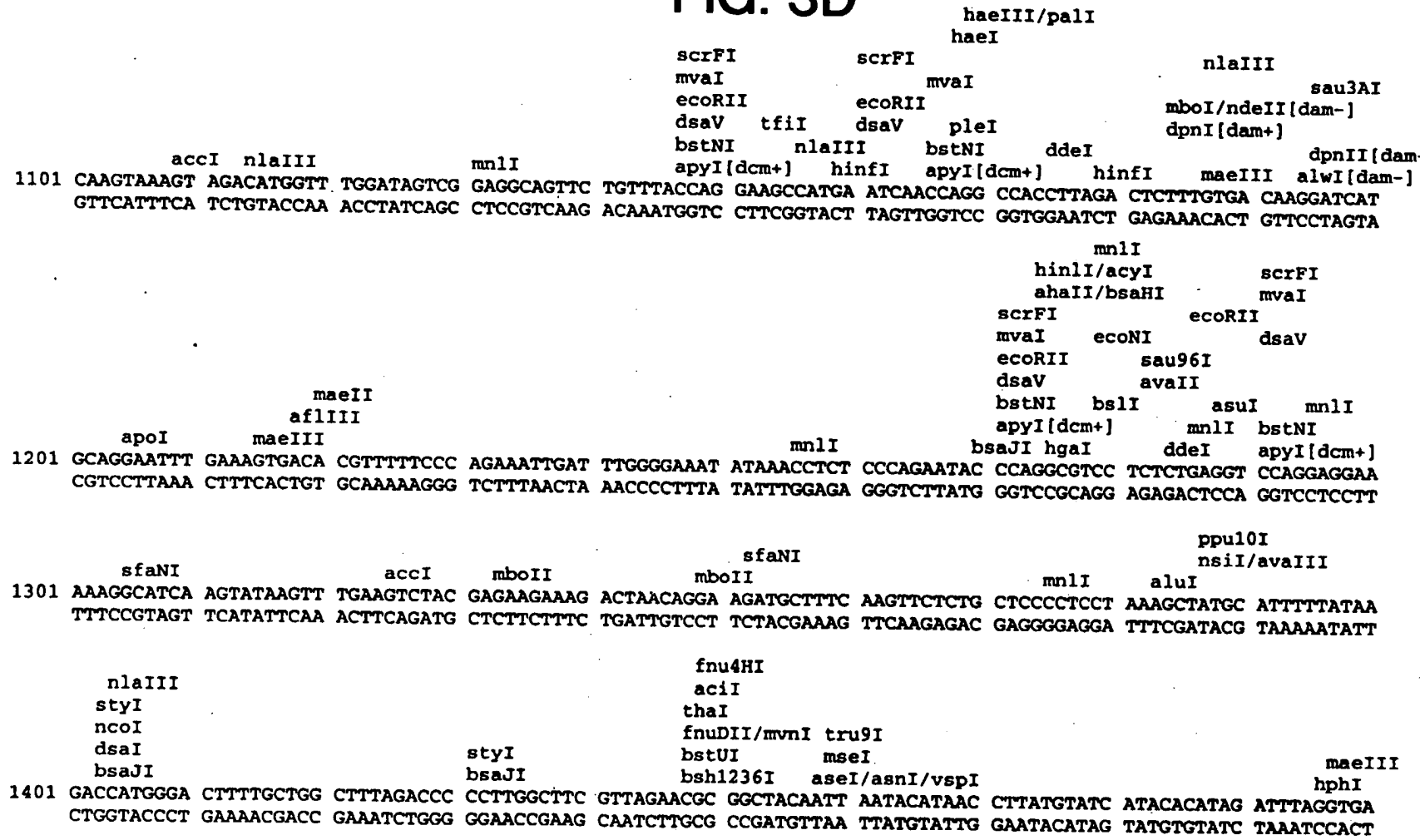


FIG. 3E

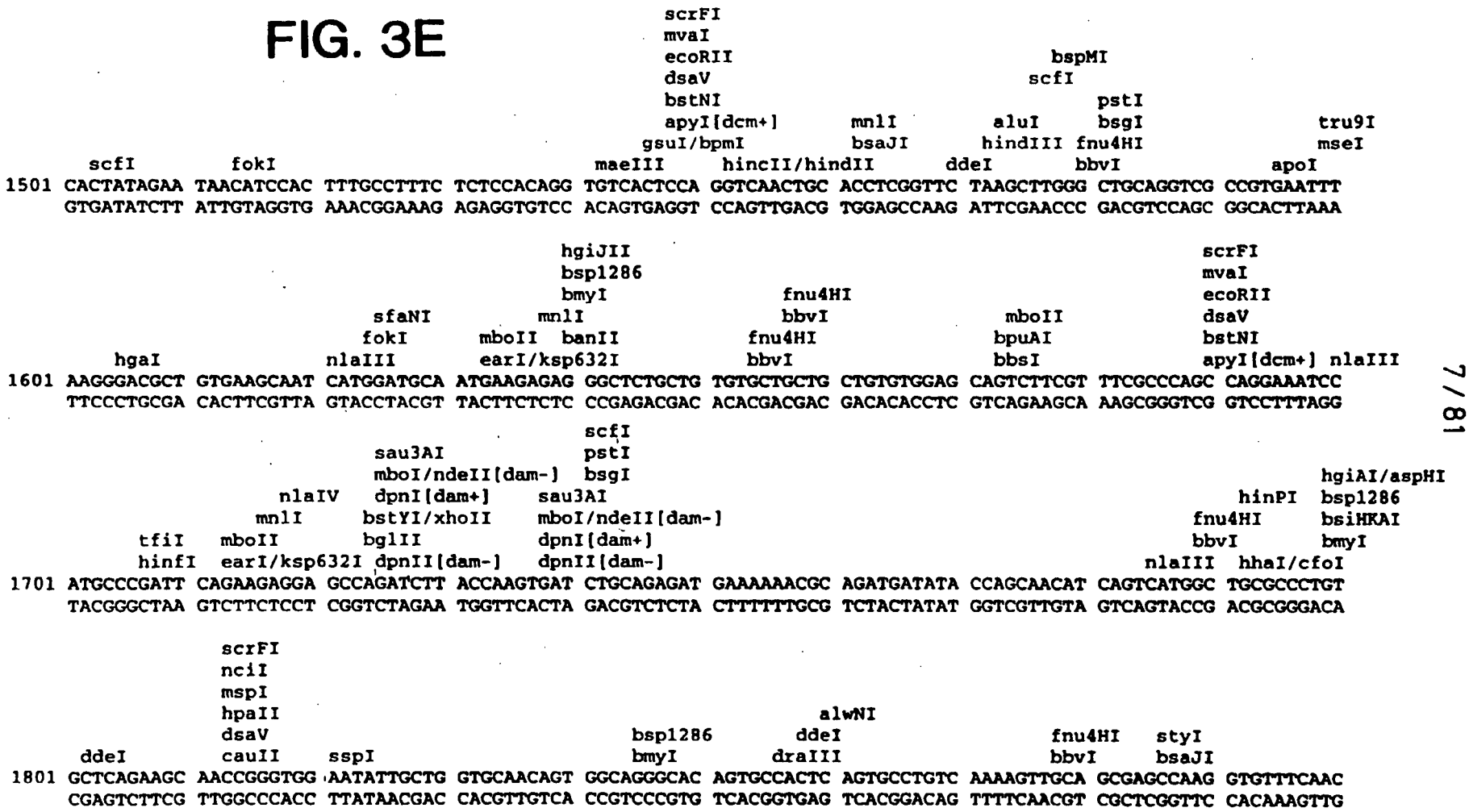


FIG. 3F

```
                                                                                                             pmlI
                                                                                                             eco72I
                                                                                                         sau96I
                                                                                                         asuI maeII
          bspMI                                                                                      scrFI
       nlaIV          sau96I                                                                         mvaI haeIII/palI
       hgiCI          haeIII/palI                                                                    ecoRII
       banI           asuI   rsaI                          bsp1286                                   dsaV
      bsp1286        eco0109I/draII                        bmyI                                      bstNI   bsaAI
      bmyI          alwNI    csp6I ddeI                 bsrI      bcgI                               bsaJI   bbrPI
                                                                                                     apyI[dcm+]
1901 GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT
     CCCCCGTGGA CGGTCGTCCG GGACATGAAG AGTCTAAAGC ACACGGTCAC GGGGCTTCCT AAACGACCCT TCACGACACT TTATCTATGG TCCCGGTGCA

               scrFI                                     hinPI
               mvaI                                            hhaI/cfoI
               ecoRII                                   nlaIV    hgiAI/aspHI
               dsaV                                     narI     bsp1286                thaI
               bstNI                  pmlI  hgiAI/aspHI      kasI     bsiHKAI                fnuDII/mvnI
               bsaJI                  eco72I                 hinlI/acyI                      bstUI sau96I
           sau96I               bsaAI bsp1286           hgiCI    bmyI                  hinPI  haeIII/palI
           avaII                bbrPI bsiHKAI           haeII    apaLI/snoI            hhaI/cfoI
           asuI     sfaNI   scfI  bsp1286  bmyI     aciI     banI     alw44I/snoI        fnu4HI     asuI        bslI
         mnlI  apyI[dcm+] aluI    bmyI maeII      nspBII     ahaII/bsaHI     bsrI        bbvI bsh1236I        bslI
2001 GCTACGAGGA CCAGGGCATC AGCTACAGGG GCACGTGGAG CACAGCGGAG AGTGGCGCCG AGTGCACCAA CTGGAACAGC AGCGCGTTGG CCCAGAAGCC
     CGATGCTCCT GGTCCCGTAG TCGATGTCCC CGTGCACCTC GTGTCGCCTC TCACCGCGGC TCACGTGGTT GACCTTGTCG TCGCGCAACC GGGTCTTCGG

                                           scrFI
                                           mvaI
                                           ecoRII
                                           dsaV                                  pleI        scrFI
                                           bstNI                                     bsmAI         mvaI
                                           bsaJI                                taqI[dam-]         ecoRII
                        hinlI/acyI         apyI[dcm+]                          sau3AI hinfI        dsaV
             aciI haeIII/palI            haeIII/palI           scfI            mboI/ndeII[dam-]    bstNI
       nspBII     haeI  hgaI bstXI      sau96I          pstI           dpnI[dam+]          apyI[dcm+]      tru9I
     scfI aciI    mnlI  ahaII/bsaHI     asuI     nlaIV         bsgI           dpnII[dam-]       bsaJI      maeII mseI
2101 CTACAGCGGG CGGAGGCCAG ACGCCATCAG GCTGGGCCTG GGGAACCACA ACTACTGCAG AAACCCAGAT CGAGACTCAA AGCCCTGGTG CTACGTCTTT
     GATGTCGCCC GCCTCCGGTC TGCGGTAGTC CGACCCGGAC CCCTTGGTGT TGATGACGTC TTTGGGTCTA GCTCTGAGTT TCGGGACCAC GATGCAGAAA
```

FIG. 3G

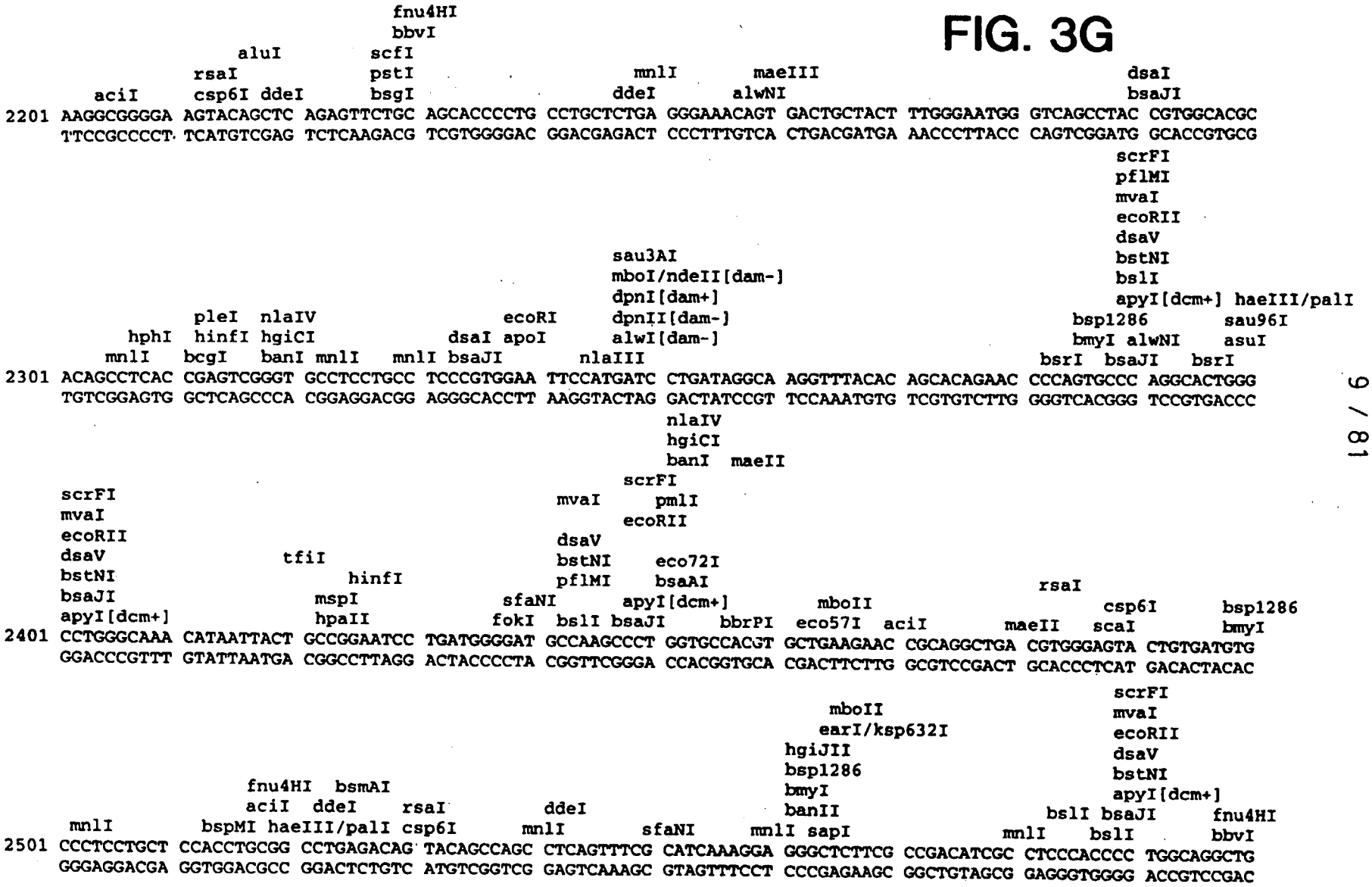

fnu4HI
bbvI
aluI
scfI
rsaI
pstI
mnlI
maeIII
dsaI
aciI
csp6I ddeI
bsgI
ddeI
alwNI
bsaJI
2201 AAGGCGGGGA AGTACAGCTC AGAGTTCTGC AGCACCCCTG CCTGCTCTGA GGGAAACAGT GACTGCTACT TTGGGAATGG GTCAGCCTAC CGTGGCACGC
TTCCGCCCCT TCATGTCGAG TCTCAAGACG TCGTGGGGAC GGACGAGACT CCCTTTGTCA CTGACGATGA AACCCTTACC CAGTCGGATG GCACCGTGCG
scrFI
pflMI
mvaI
ecoRII
dsaV
sau3AI
bstNI
mboI/ndeII[dam-]
bslI
dpnI[dam+]
apyI[dcm+] haeIII/palI
pleI nlaIV
ecoRI
dpnII[dam-]
bsp1286
sau96I
hphI hinfI hgiCI
dsaI apoI
alwI[dam-]
bmyI alwNI
asuI
mnlI bcgI banI mnlI mnlI bsaJI
nlaIII
bsrI bsaJI bsrI
2301 ACAGCCTCAC CGAGTCGGGT GCCTCCTGCC TCCCGTGGAA TTCCATGATC CTGATAGGCA AGGTTTACAC AGCACAGAAC CCCAGTGCCC AGGCACTGGG
TGTCGGAGTG GCTCAGCCCA CGGAGGACGG AGGGCACCTT AAGGTACTAG GACTATCCGT TCCAAATGTG TCGTGTCTTG GGGTCACGGG TCCGTGACCC
nlaIV
hgiCI
banI maeII
scrFI
scrFI
mvaI
pmlI
mvaI
ecoRII
ecoRII
dsaV
dsaV
tfiI
bstNI
eco72I
bstNI
hinfI
pflMI
bsaAI
bsaJI
rsaI
mspI
sfaNI
apyI[dcm+]
mboII
csp6I
bsp1286
apyI[dcm+]
hpaII
fokI bslI bsaJI
bbrPI eco57I aciI
maeII scaI
bmyI
2401 CCTGGGCAAA CATAATTACT GCCGGAATCC TGATGGGGAT GCCAAGCCCT GGTGCCACGT GCTGAAGAAC CGCAGGCTGA CGTGGGAGTA CTGTGATGTG
GGACCCGTTT GTATTAATGA CGGCCTTAGG ACTACCCCTA CGGTTCGGGA CCACGGTGCA CGACTTCTTG GCGTCCGACT GCACCCTCAT GACACTACAC
scrFI
mboII
mvaI
earI/ksp632I
ecoRII
hgiJII
dsaV
bsp1286
bstNI
fnu4HI bsmAI
bmyI
apyI[dcm+]
aciI ddeI rsaI
ddeI
banII
bslI bsaJI
fnu4HI
mnlI
bspMI haeIII/palI csp6I
mnlI
sfaNI
mnlI sapI
mnlI
bslI
bbvI
2501 CCCTCCTGCT CCACCTGCGG CCTGAGACAG TACAGCCAGC CTCAGTTTCG CATCAAAGGA GGGCTCTTCG CCGACATCGC CTCCCACCCC TGGCAGGCTG
GGGAGGACGA GGTGGACGCC GGACTCTGTC ATGTCGGTCG GAGTCAAAGC GTAGTTTCCT CCCGAGAAGC GGCTGTAGCG GAGGGTGGGG ACCGTCCGAC

FIG. 3H

```
                                mspI
                                hpaII                                                                 scrFI
                                bslI                                                                  mvaI
                               scrFI                                                                  ecoRII
                               nciI      nlaIV                                                        dsaV
                               dsaV    aciI                        aluI       tfiI       aciI         bstNI
             bslI      mnlI    cauII bsrBI   bslI aciI             alwNI      hinfI     fnu4HI        apyI[dcm+]
2601 CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA
     GGTAGAAACG GTTCGTGTCC TCCAGCGGGC CTCTCGCCAA GGACACGCCC CCGTATGAGT AGTCGAGGAC GACCTAAGAG AGACGGCGGG TGACGAAGGT

                                                               scrFI
                                                               mvaI
                                                               ecoRII
                                                               dsaV
                                                               bstNI
                                                               apyI[dcm+]
                                                    scrFI     bslI
                                   sau3AI           nciI      bsaJI
                                   mboI/ndeII[dam-] mspI   sau96I
                                   dpnI[dam+]       hpaII  nlaIV
                                   dpnII[dam-]      dsaV   avaII       mnlI
        mnlI   aciI   bslI      hphI[dam-]          cauII  asuI      mnlI        apoI      taqI
2701 GGAGAGGTTT CCGCCCCACC ACCTGACGGT GATCTTGGGC AGAACATACC GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT
     CCTCTCCAAA GGCGGGGTGG TGGACTGCCA CTAGAACCCG TCTTGTATGG CCCACCAGGG ACCGCTCCTC CTCGTCTTTA AACTTCAGCT TTTTATGTAA

                                                    scfI                             scrFI
                                                    pstI                             mvaI
                                                   fnu4HI                            ecoRII
                                                   bbvI pvuII                        dsaV
                                                fnu4HI fnu4HI                        bstNI            aciI
                                                 bbvI  bbvI                      bsp1286     fnu4HI sau96I
             ecoRI                         hinPI bsgI aluI     tfiI    nspBII  bsaJI    bbvI   avaII
             apoI taqI                     hhaI/cfoI nspBII     hinfI   aciI bmyI apyI[dcm+]    asuI
2801 GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT GCAGCTGAAA TCGGATTCGT CCCGCTGTGC CCAGGAGAGC AGCGTGGTCC
     CAGGTATTCC TTAAGCTACT ACTGTGAATG CTGTTACTGT AACGCGACGA CGTCGACTTT AGCCTAAGCA GGGCGACACG GGTCCTCTCG TCGCACCAGG
```

FIG. 3I

```
                                                                aluI
                                                               sstI
                                    pvuII                      sacI
                       mspI      scfI                          hgiJII
                       hpaII     pstI  fnu4HI                  hgiAI/aspHI
                      scrFI    bspMI   bbvI                    ecl136II            haeIII/palI
                      nciI   sau96I nspBII                     bsp1286            stuI
                      dsaV   avaII fnu4HI      bsrI            bsiHKAI            haeI
                      cauII  asuI  bbvI    mspI                bmyI    mspI              mnlI
      draIII    bslI bsaJI aciI  bsgI aluI hpaII               banII   hpaII          nlaIII      bsmAI
2901 GCACTGTGTG CCTTCCCCCG GCGGACCTGC AGCTGCCGGA CTGGACGGAG TGTGAGCTCT CCGGCTACGG CAAGCATGAG GCCTTGTCTC CTTTCTATTC
     CGTGACACAC GGAAGGGGGC CGCCTGGACG TCGACGGCCT GACCTGCCTC ACACTCGAGA GGCCGATGCC GTTCGTACTC CGGAACAGAG GAAAGATAAG

                                                     fnu4HI
                                                   nspBII
        fnu4HI                                     aciI                                             nlaIII
        aciI                          rsaI fokI   fnu4HI                    tru9I        hphI      nspI
      bsrBI eco57I mnlI nlaIII       csp6I    alwNI bbvI                   mseI       maeIII     nspHI        gsuI/bpmI
3001 GGAGCGGCTG AAGGAGGCTC ATGTCAGACT GTACCCATCC AGCCGCTGCA CATCACAACA TTTACTTAAC AGAACAGTCA CCGACAACAT GCTGTGTGCT
     CCTCGCCGAC TTCCTCCGAG TACAGTCTGA CATGGGTAGG TCGGCGACGT GTAGTGTTGT AAATGAATTG TCTTGTCAGT GGCTGTTGTA CGACACACGA

                         sau96I
                         nlaIV
                         haeIII/palI
                         asuI
                        sau96I
                        nlaIV scrFI                                      scrFI
                        hgiJII                                                 mvaI
                        eco0109I/draII            scrFI            ecoRII
                        bsp1286                   mvaI                   dsaV
                        bsp120I                   ecoRII           bstNI
                        bmyI  mvaI                dsaV        sau96I
                        banII ecoRII        bstNI             nlaIV                   aciI
                      aciI    dsaV          bsaJI             haeIII/palI            fnu4HI
                   fnu4HI     bstNI      hinlI/acyI      tfiI      asuI apyI[dcm+]           haeIII/palI
                   aciI asuI  apyI[dcm+]     hgaI     apyI[dcm+]       eco0109I/draII           eaeI
      bsmAI      bsrBI  apaI bsaJI           ahaII/bsaHI     hinfI   mnlI  bsaJI                cfrI   nlaIII
3101 GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA GGGCGATTCG GGAGGCCCCC TGGTGTGTCT GAACGATGGC CGCATGACTT
     CCTCTGTGAG CCTCGCCGCC CGGGGTCCGT TTGAACGTGC TGCGGACGGT CCCGCTAAGC CCTCCGGGGG ACCACACAGA CTTGCTACCG GCGTACTGAA
```

FIG. 3J

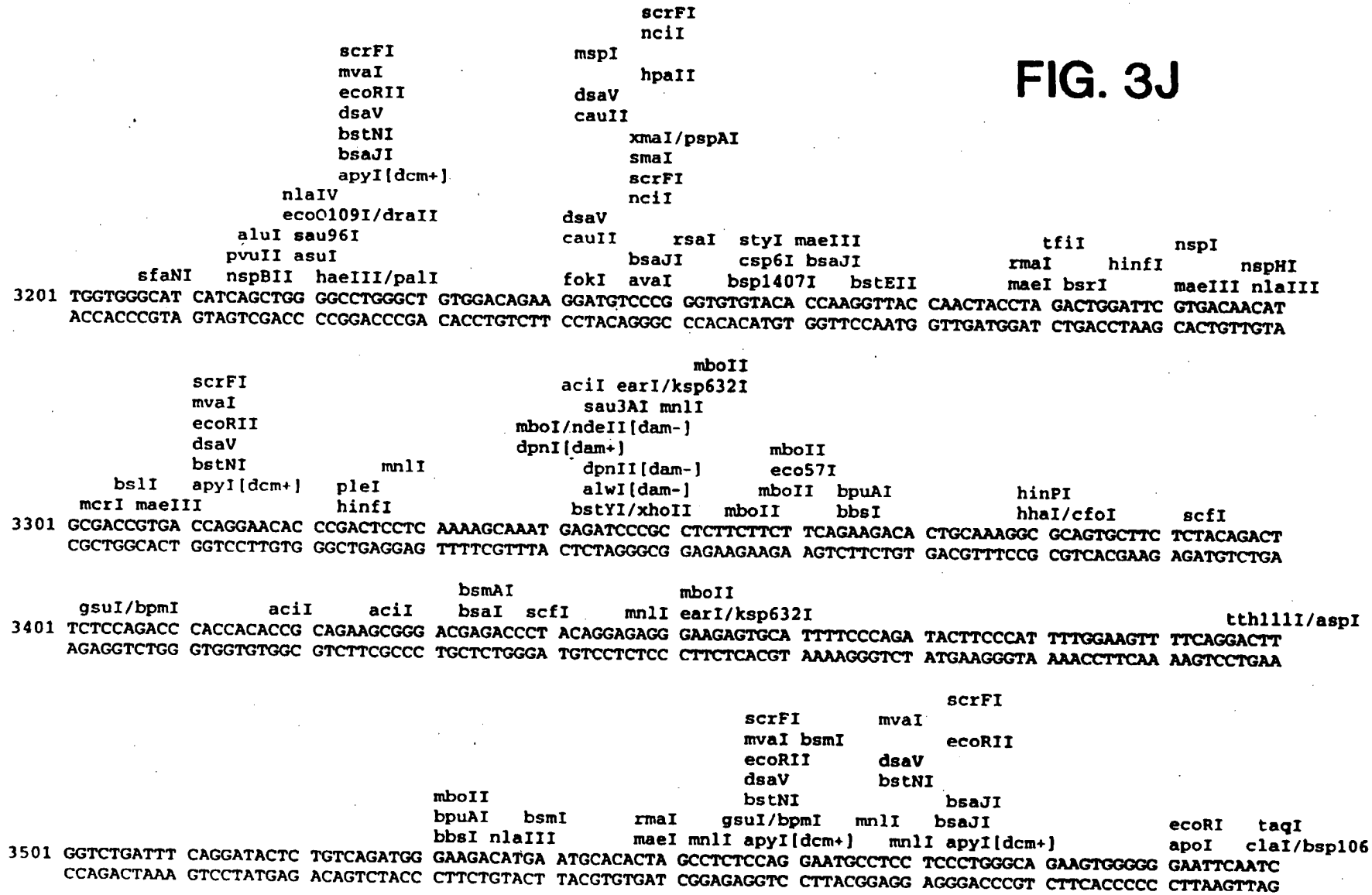


FIG. 3K

```
                styI
              aciI
             fnu4HI   sau96I
             bglI nlaIII
            sfiI ncoI haeIII/palI
            haeIII/palI                    aluI
           eaeI  dsaI asuI               fnu4HI
           cfrI  bsaJI                   bbvI          maeIII              sfaNI  apoI                                  bsmI
3601 GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
     CTACCGGCGG TACCGGGTTG AACAAATAAC GTCGAATATT ACCAATGTTT ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT

                                                                sau3AI
                                                                mboI/ndeII[dam-]
                                                                dpnI[dam+]
                                                                dpnII[dam-]
                                                               pvuI/bspCI
                                                               mcrI
                                                              taqI[dam-]  tru9I
                                                             claI/bsp106[dam-]              styI
                                                            sau3AI          mseI       fnu4HI    haeI
                                                            mboI/ndeII[dam-]           bbvI  ncoI
                                                            dpnI[dam+] xmnI        hinPI    dsaI haeIII/palI
      rmaI                                                  dpnII[dam-]    aseI/asnI/vspI    bsaJI
      maeI                                                nlaIII alwI[dam-]  asp700      hhaI/cfoI nlaIII           mnlI
3701 TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA
     AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT AGCTAGCCCT TAATTAAGCC GCGTCGTGGT ACCGGACTTT ATTGGAGACT

                      rsaI                                                                    nlaIV
                      csp6I                                                                 scrFI
                     nlaIV                                                                  mvaI
                     kpnI                                                                   ecoRII
                     hgiCI                                                                  dsaV
                     banI                              aluI                                 bstNI
                     asp718     mnlI                   pvuII                                apyI[dcm+]
      mnlI           acc65I   ddeI aciI                nspBII                             bsaJI
3801 AAGAGGAACT TGGTTAGGTA CCTTCTGAGG CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
     TTCTCCTTGA ACCAATCCAT GGAAGACTCC GCCTTTCTTG GTCGACACCT TACACACAGT CAATCCCACA CCTTTCAGGG GTCCGAGGGG TCGTCCGTCT
```

FIG. 3L

```
                     sfaNI                   scrFI      scrFI       nlaIV                 sfaNI
                   ppu10I                    mvaI           mvaI                                     ppu10I
                   nsiI/avaIII               ecoRII     ecoRII                          nsiI/avaIII
                  nlaIII                     dsaV       dsaV                           nlaIII
                 sphI                        bstNI           bstNI                                sphI
                 nspI                        apyI[dcm+]      apyI[dcm+]                           nspI
                 nspHI                      sexAI           bsaJI                                 nspHI
3901 AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT
     TCATACGTTT CGTACGTAGA GTTAATCAGT CGTTGGTCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA

                                                                                          nlaIII
                                                                                         styI
                                                                                         ncoI
                                              aciI                                bslI   dsaI
                     aciI         aciI   fokI                 aciI bsrI   aciI    aciI   bsaJI
4001 CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC TGACTAATTT TTTTTATTTA
     GTCGTTGGTA TCAGGGCGGG GATTGAGGCG GGTAGGGCGG GGATTGAGGC GGGTCAAGGC GGGTAAGAGG CGGGGTACCG ACTGATTAAA AAAAATAAAT

                                                                                 rmaI
                                                                                styI
                     aciI                                                       bsaJI
                    fnu4HI                                                      blnI
                   sfiI                                                         avrII
                   haeIII/palI                                                haeIII/palI               tru9I
              bsaJI bglI         ddeI                                         stuI                     hpaI
            haeIII/palI mnlI haeIII/palI                      mnlI            haeI                     hincII/hindII
          mnlI    mnlI    bsaJI  mnlI  aluI               mnlI                mnlI maeI            aluI mseI  aluI
4101 TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTGTT AACAGCTTGG
     ACGTCTCCGG CTCCGGCGGA GCCGGAGACT CGATAAGGTC TTCATCACTC CTCCGAAAAA ACCTCCGGAT CCGAAAACGT TTTTCGACAA TTGTCGAACC

                                                 scrFI
                                                 mvaI
                                                 ecoRII
           haeIII/palI                           dsaV
          eaeI                                   bstNI                                                    aluI
          cfrI                       bsrI        apyI[dcm+]         tru9I        fnu4HI                  pvuII
        bsrI               maeII maeIII         bsaJI      maeIII   mseI         bbvI   fokI             nspBII
4201 CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG
     GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC
```

FIG. 3M

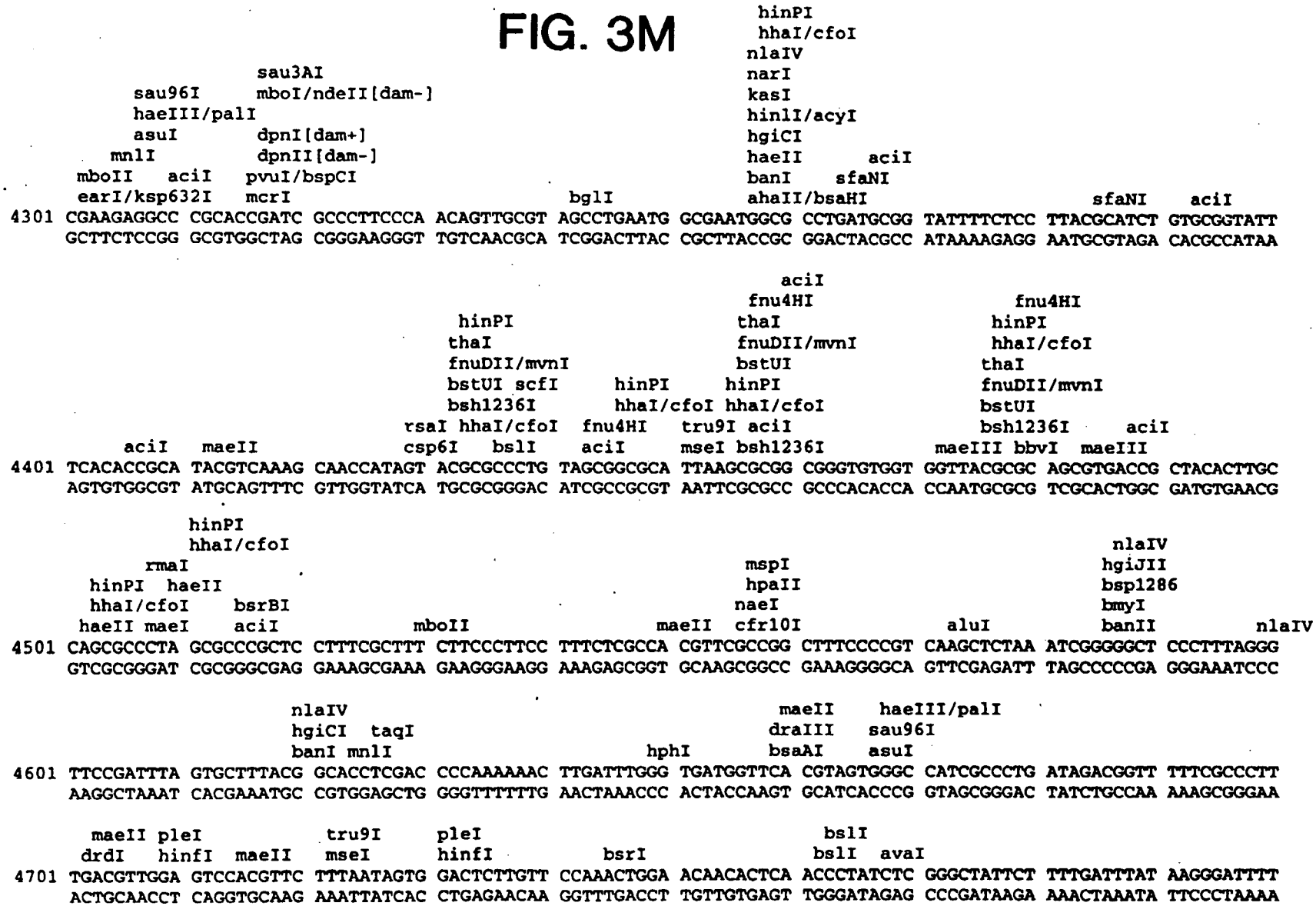
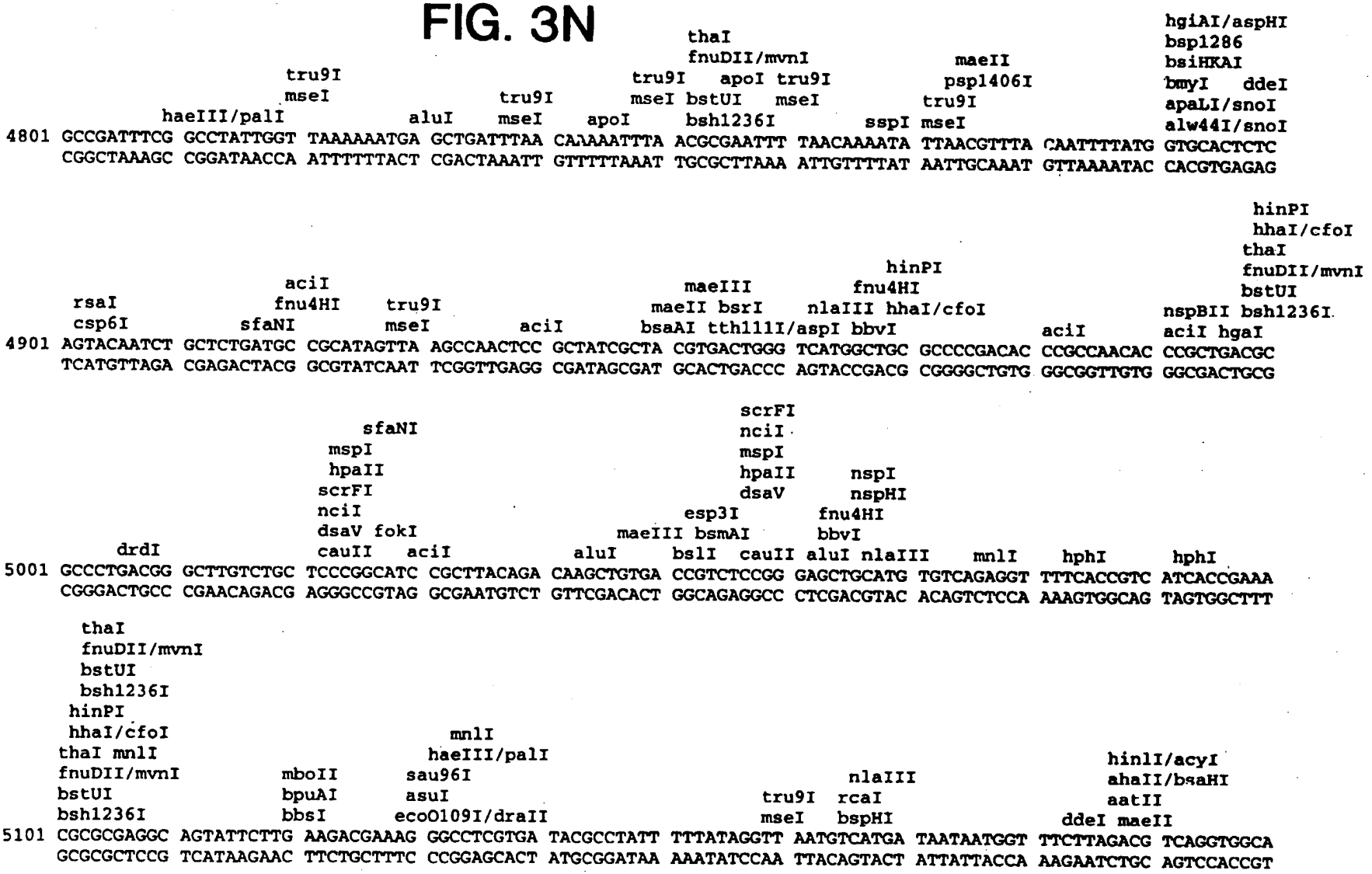

hinPI
hhaI/cfoI
nlaIV
narI
kasI
hinlI/acyI
hgiCI
haeII aciI
banI sfaNI
ahaII/bsaHI sfaNI aciI
sau3AI
sau96I mboI/ndeII[dam-]
haeIII/palI
asuI dpnI[dam+]
mnlI dpnII[dam-]
mboII aciI pvuI/bspCI
earI/ksp632I mcrI bglI
4301 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGT AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT
GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCA TCGGACTTAC CGCTTACCGC GGACTACGCC ATAAAAGAGG AATGCGTAGA CACGCCATAA
aciI
fnu4HI
thaI
fnuDII/mvnI
bstUI
hinPI
thaI
fnuDII/mvnI
bstUI scfI
bsh1236I
hinPI
hhaI/cfoI
hinPI
hhaI/cfoI
rsaI hhaI/cfoI
csp6I
fnu4HI
bslI
aciI
tru9I aciI
mseI bsh1236I
fnu4HI
hinPI
hhaI/cfoI
thaI
fnuDII/mvnI
bstUI
bsh1236I aciI
aciI maeII
maeIII bbvI maeIII
4401 TCACACCGCA TACGTCAAAG CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC
AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGCGCGGGAC ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG
hinPI
hhaI/cfoI
rmaI
hinPI haeII
hhaI/cfoI bsrBI
haeII maeI aciI
mboII
mspI
hpaII
naeI
maeII cfr10I
aluI
nlaIV
hgiJII
bsp1286
bmyI
banII nlaIV
4501 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG
GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT TAGCCCCCGA GGGAAATCCC
nlaIV
hgiCI taqI
banI mnlI
hphI
maeII haeIII/palI
draIII sau96I
bsaAI asuI
4601 TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT
AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG AACTAAACCC ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA
maeII pleI
drdI hinfI maeII
tru9I
mseI
pleI
hinfI
bsrI
bslI
bslI avaI
4701 TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTTGATTTAT AAGGGATTTT
ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AAACTAAATA TTCCCTAAAA

FIG. 3N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fnuDII/mvnI
hgiAI/aspHI
bsp1286
bsiHKAI
bmyI ddeI
apaLI/snoI
alw44I/snoI
tru9I
mseI
haeIII/palI
aluI
apoI
bsh1236I
maeII
psp1406I
sspI
4801 GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC
CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG
hinPI
hhaI/cfoI
thaI
fnuDII/mvnI
bstUI
nspBII bsh1236I
aciI
hgaI
rsaI
csp6I
aciI
fnu4HI
sfaNI
tru9I
mseI
maeIII
maeII bsrI
bsaAI tth111I/aspI
hinPI
fnu4HI
nlaIII hhaI/cfoI
bbvI
4901 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC
TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG
scrFI
nciI
sfaNI
mspI
hpaII
scrFI
nciI
dsaV fokI
esp3I
maeIII bsmAI
nspI
nspHI
fnu4HI
bbvI
drdI
cauII aciI
aluI
bslI cauII aluI nlaIII
mnlI
hphI
hphI
5001 GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA
CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT
thaI
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
mnlI
thaI mnlI
haeIII/palI
fnuDII/mvnI
mboII
sau96I
hinlI/acyI
ahaII/bsaHI
bstUI
bpuAI
asuI
nlaIII
aatII
bsh1236I
bbsI
eco0109I/draII
tru9I rcaI
mseI bspHI
ddeI maeII
5101 CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA
GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT

FIG. 3O

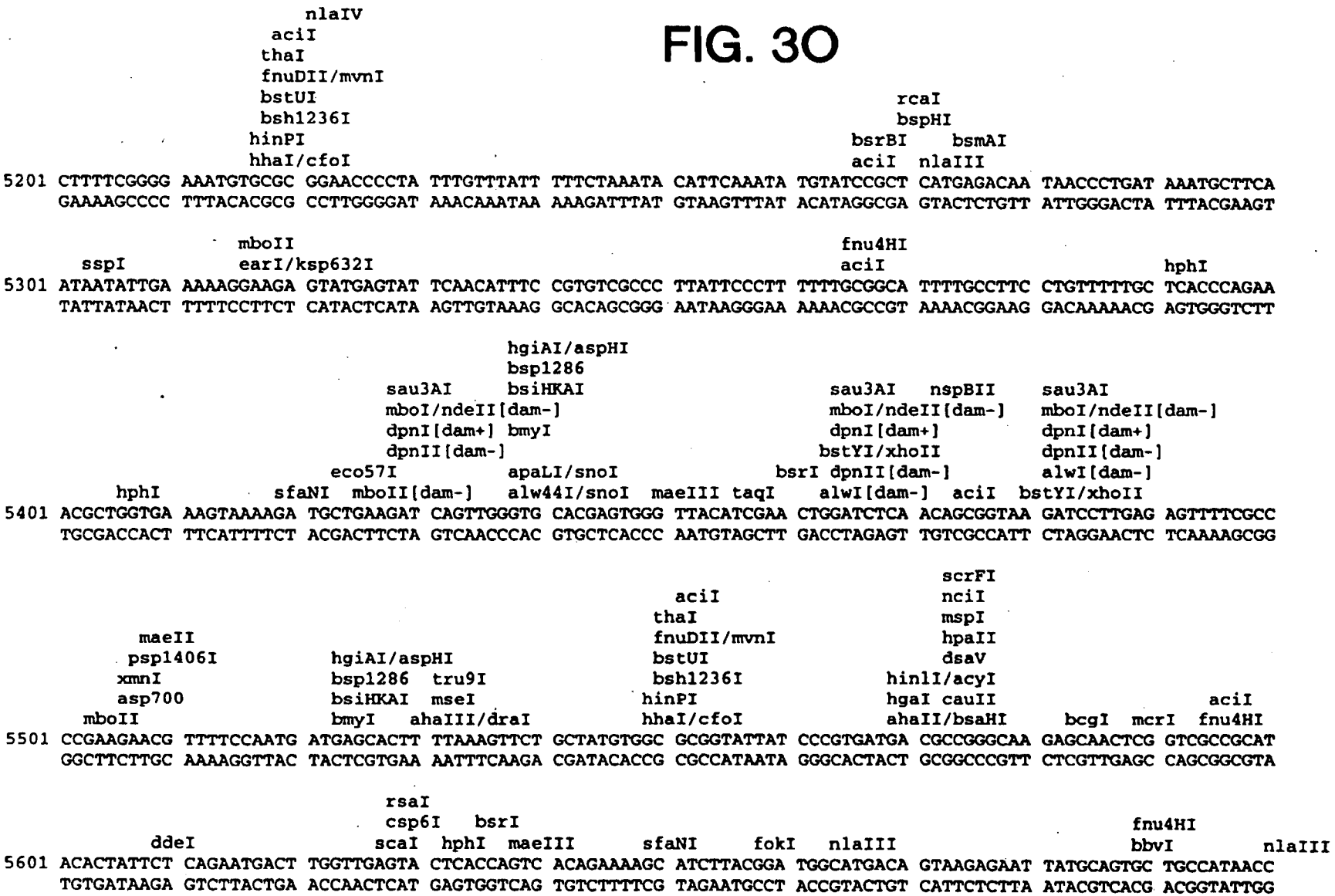
nlaIV
aciI
thaI
fnuDII/mvnI
bstUI
bsh1236I
hinPI
hhaI/cfoI
rcaI
bspHI
bsrBI bsmAI
aciI nlaIII
5201 CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT
mboII
sspI earI/ksp632I
fnu4HI
aciI
hphI
5301 ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT
hgiAI/aspHI
bsp1286
sau3AI bsiHKAI
mboI/ndeII[dam-]
dpnI[dam+] bmyI
dpnII[dam-]
sau3AI nspBII
mboI/ndeII[dam-]
dpnI[dam+]
bstYI/xhoII
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
eco57I apaLI/snoI
bsrI dpnII[dam-]
alwI[dam-]
hphI sfaNI mboII[dam-] alw44I/snoI maeIII taqI alwI[dam-] aciI bstYI/xhoII
5401 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC
TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG
scrFI
aciI nciI
thaI mspI
maeII fnuDII/mvnI hpaII
psp1406I hgiAI/aspHI bstUI dsaV
xmnI bsp1286 tru9I bsh1236I hinlI/acyI
asp700 bsiHKAI mseI hinPI hgaI cauII aciI
mboII bmyI ahaIII/draI hhaI/cfoI ahaII/bsaHI bcgI mcrI fnu4HI
5501 CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA
rsaI
csp6I bsrI
fnu4HI
ddeI scaI hphI maeIII sfaNI fokI nlaIII bbvI nlaIII
5601 ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG

FIG. 3P


sau96I
avaII
sau3AI asuI
nlaIII
haeIII/palI
mboI/ndeII[dam-]
sau3AI maeIII
eaeI
dpnI[dam+]
mboI/ndeII[dam-]
cfrI
dpnII[dam-]
dpnI[dam+]
fnu4HI
pvuI/bspCI
dpnII[dam-]
aciI
mcrI mnlI
aluI aciI
nlaIII alwI[dam-]
5701 ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC
TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG
hinPI
mspI
hhaI/cfoI
sau3AI nlaIV
mstI
mboI/ndeII[dam-] aluI
aviII/fspI bsrI
dpnI[dam+] hpaII
fnu4HI maeII tru9I
dpnII[dam-] bsaWI
maeIII sfaNI bbvI psp1406I mseI
5801 TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC
AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG
mspI
hpaII
bglI
scrFI
sau96I
aluI nciI tru9I fokI sau96I haeIII/palI
rmaI dsaV mseI bsrI aciI avaII hinPI asuI mspI
maeI cauII aseI/asnI/vspI mnlI asuI hhaI/cfoI hpaII
5901 TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG
mspI thaI
hpaII fnuDII/mvnI haeIII/palI
cfr10I bstUI sau96I
nlaIV hphI bsmAI aciI fnu4HI nlaIV
gsuI/bpmI bsaI bsh1236I bbvI bsrI asuI mnlI
6001 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA
ddeI
sau3AI nlaIV
pleI
mboI/ndeII[dam-]
hinfI
dpnI[dam+] hgiCI tru9I
eam1105I fokI dpnII[dam-] banI mnlI mseI maeIII
6101 ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA
TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT

FIG. 3Q

```
                                                             rmaI      sau3AI
                                                         sau3AI hphI   mboI/ndeII[dam-]
                                                         mboI/ndeII[dam-]
                                                         dpnI[dam+]    dpnI[dam+]
                                                         dpnII[dam+]   dpnII[dam-]
                             tru9I                tru9I bstYI/xhoII    alwI[dam-]         nlaIII              maeII
                             mseI           tru9I mseI  alwI[dam-]   bstYI/xhoII         rcaI              tru9I
                            ahaIII/draI     mseI ahaIII/draI maeI  mboII[dam-]           bspHI             mseI
6201 CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT
     GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA

                                                       sau3AI
                                                       mboI/ndeII[dam-]
                                                       dpnI[dam+]  sau3AI
                                                       dpnII[dam-] mboI/ndeII[dam-] thaI
                                                      bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                              sau3AI  alwI[dam-]   dpnII[dam-]      bstUI
                                              mboI/ndeII[dam-]     alwI[dam-]       bsh1236I
                        hgaI                  dpnI[dam+] mboII[dam-]               hinPI      fnu4HI
                    ddeI                      dpnII[dam-]         bstYI/xhoII      hhaI/cfoI  bbvI
6301 GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA
     CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT

                                        sau3AI
                                        mboI/ndeII[dam-]
                                        dpnI[dam+]
                                        dpnII[dam-]
                                       alwI[dam-]
                    aciI               mspI                                bsrI                hinPI
          aciI    nspBII               hpaII       aluI                  maeIII      eco57I    hhaI/cfoI
6401 AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT
     TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA

                                                                                                         fnu4HI
                                                                                                alwNI    bbvI
         rmaI                haeIII/palI                                                       bsrI  fnu4HI
         maeI      bslI     haeI                  scfI    aciI         mnlI               maeIII     bbvI    bsrI
6501 CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
     GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA
```

FIG. 3R

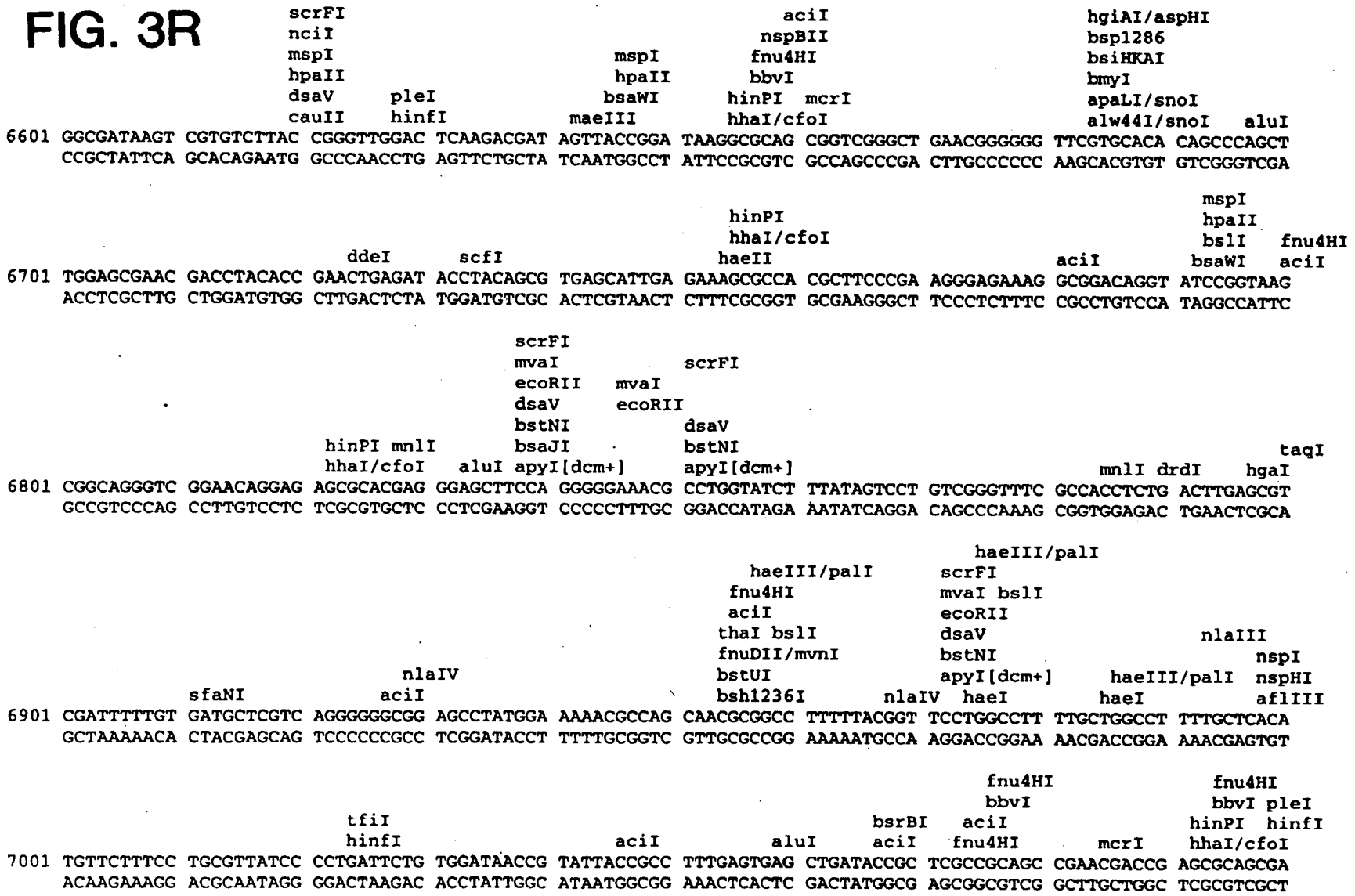
scrFI
nciI
mspI
hpaII
dsaV
cauII
pleI
hinfI
mspI
hpaII
bsaWI
maeIII
aciI
nspBII
fnu4HI
bbvI
hinPI
mcrI
hhaI/cfoI
hgiAI/aspHI
bsp1286
bsiHKAI
bmyI
apaLI/snoI
alw44I/snoI
aluI
6601 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT
CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA
hinPI
hhaI/cfoI
mspI
hpaII
bslI
fnu4HI
ddeI
scfI
haeII
aciI
bsaWI
aciI
6701 TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC
scrFI
mvaI
ecoRII
dsaV
bstNI
bsaJI
apyI[dcm+]
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
hinPI mnlI
hhaI/cfoI
aluI
taqI
mnlI drdI
hgaI
6801 CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA
haeIII/palI
haeIII/palI
scrFI
fnu4HI
mvaI bslI
aciI
ecoRII
thaI bslI
dsaV
nlaIII
fnuDII/mvnI
bstNI
nspI
nlaIV
bstUI
apyI[dcm+]
haeIII/palI
nspHI
sfaNI
aciI
bsh1236I
nlaIV haeI
haeI
aflIII
6901 CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA
GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT
fnu4HI
bbvI
fnu4HI
bbvI pleI
tfiI
hinfI
aciI
aluI
bsrBI
aciI
aciI
fnu4HI
mcrI
hinPI hinfI
hhaI/cfoI
7001 TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA
ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT

FIG. 3S

```
                                                                          thaI
                                                                          fnuDII/mvnI
                                                                          bstUI
                                                                          bsh1236I
                                                                         hinPI
                                                                         hhaI/cfoI
                                                                        thaI
                                                                        fnuDII/mvnI
                    sapI  hinPI                                         bstUI   haeIII/palI        aluI
                    mboII hhaI/cfoI                                     bsh1236I          tru9I  pvuII
                    earI/ksp632I                         mnlI           bslI    eaeI   tfiI aseI/asnI/vspI
           mnlI  aciI    haeII                        aciI              aciI    cfrI   hinfI mseI  nspBII                 bsrI
7101 GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC
     CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG

                                                                              scrFI
                                                                              mvaI
                                                                              ecoRII
                                                                              dsaV
                                        tru9I                           nlaIV bstNI
                      hinPI             mseI      maeIII                hgiCI apyI[dcm+]               mspI
          aciI        hhaI/cfoI        aseI/asnI/vspI mnlI              banI bsaJI                     hpaII
7201 TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG
     ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC

                                                                           tru9I
                                                                           mseI
                                                                          aseI/asnI/vspI
             aciI                                                       xmnI
           bsrBI                                    aluI      nlaIII    asp700
7301 GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGAATTAA
     CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCTTAATT

>length: 7360
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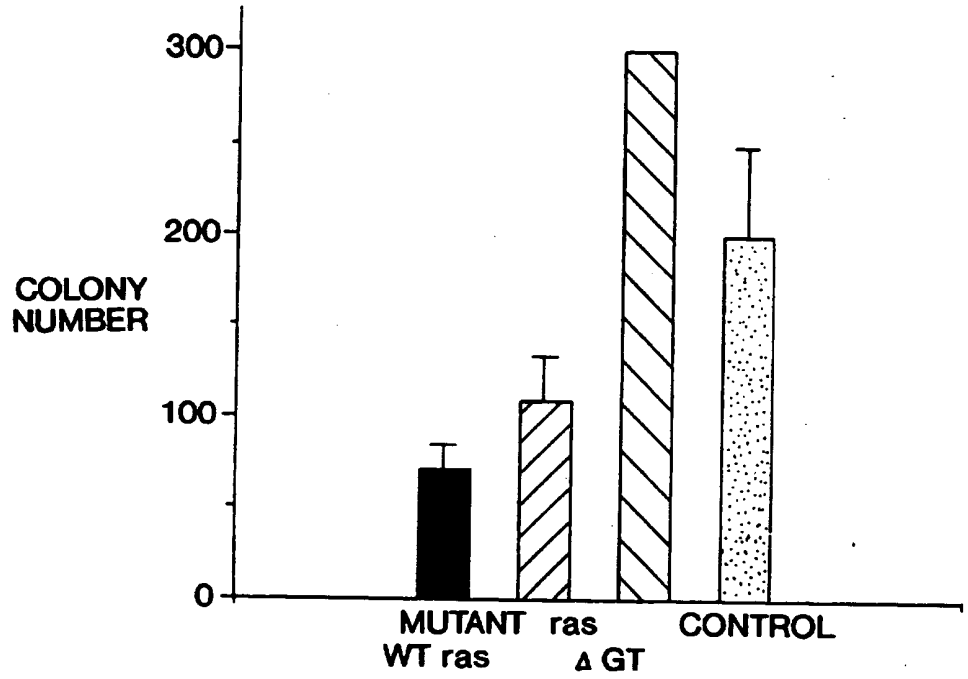

300
200
100
0
COLONY NUMBER
MUTANT ras
WT ras
Δ GT
CONTROL

FIG. 4

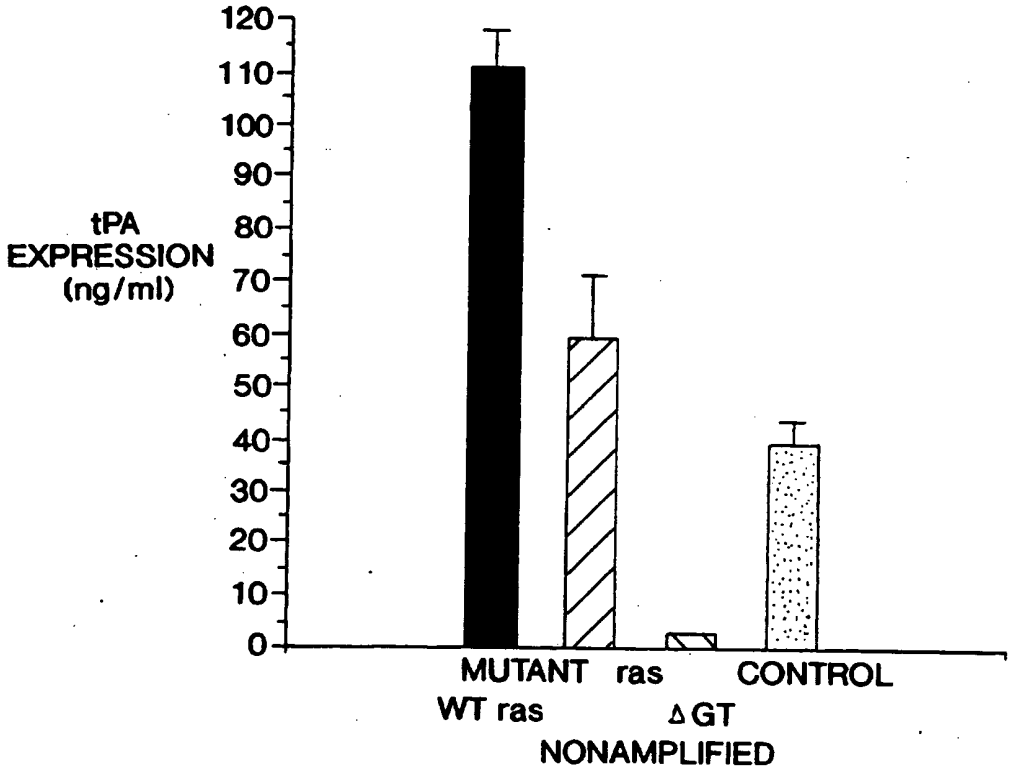

120
110
100
90
80
70
60
50
40
30
20
10
0
tPA EXPRESSION (ng/ml)
MUTANT ras
WT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5A

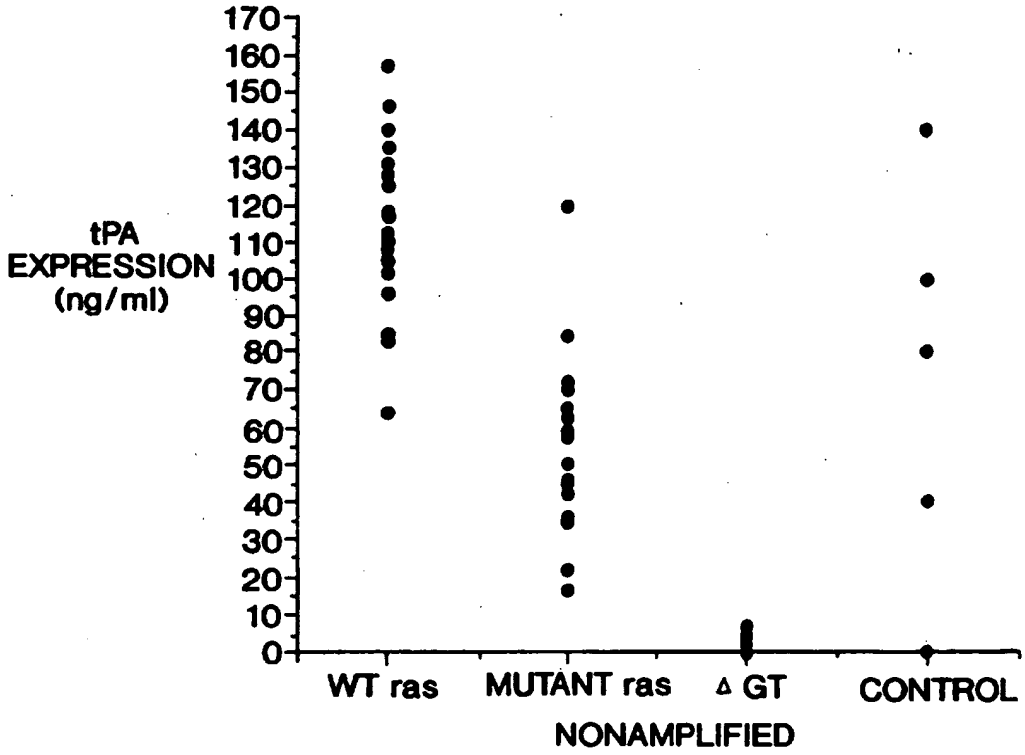

tPA
EXPRESSION
(ng/ml)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
WT ras
MUTANT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5B

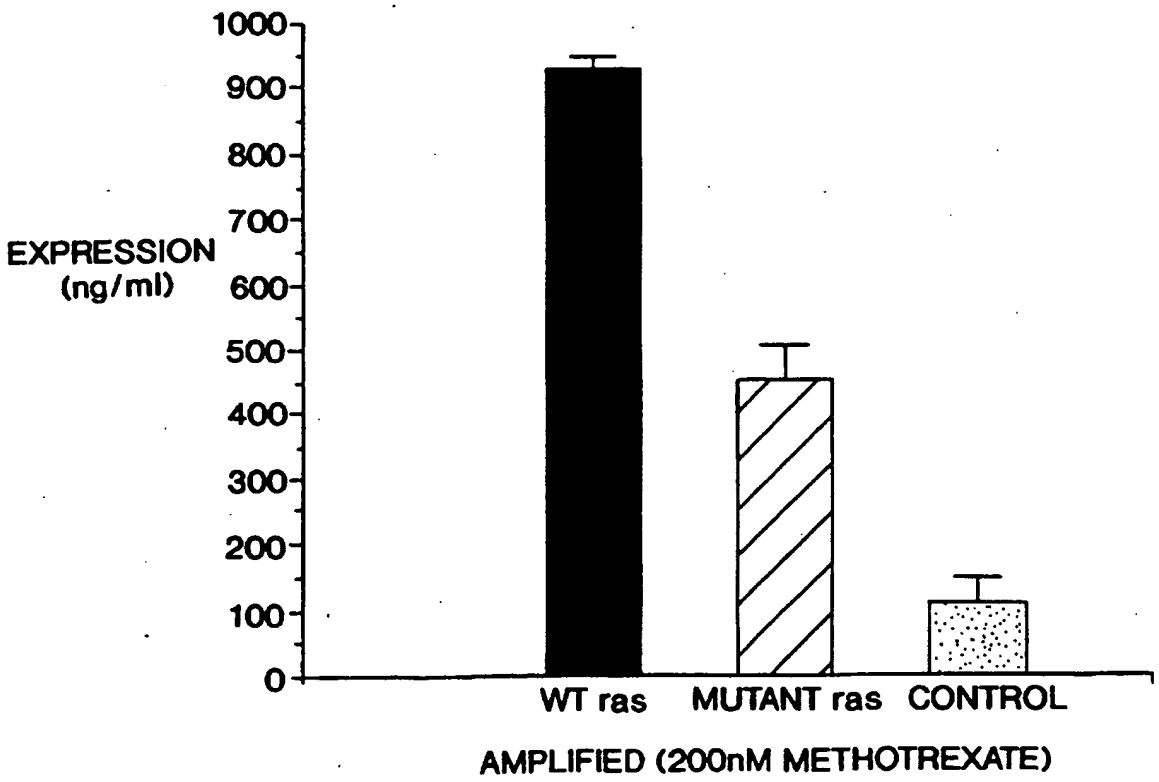

EXPRESSION
(ng/ml)
1000
900
800
700
600
500
400
300
200
100
0
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 5C

FIG. 6A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286                                                                                      thaI
       bsiHKAI                                                                                      fnuDII/mvnI
       bmyI                       rmaI    tru9I                                                     bstUI
       banII                      maeI    mseI                                                      bsh1236I
     taqI                        speI    aseI/asnI/vspI                            bslI            aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                                 maeII
                sau96I                                    hinlI/acyI
                haeIII/palI     aciI                      ahaII/bsaHI
                asuI  apyI[dcm+]                aciI      aatII          maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                       maeII
      hinlI/acyI                                                                                  hinlI/acyI
      ahaII/bsaHI                                      rsaI                        rsaI           ahaII/bsaHI
      aatII                              bglI          csp6I           ndeI       csp6I           aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                         nlaIII
         bglI  dsaV                                                                            styI
        sau96I bstNI           rsaI                                          maeII                  ncoI
        haeIII/palI            csp6I                              rsaI    snaBI                   dsaI hphI  aciI
        asuI   apyI[dcm+]    bsrI  nlaIII                            csp6I   bsaAI                   bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 6B

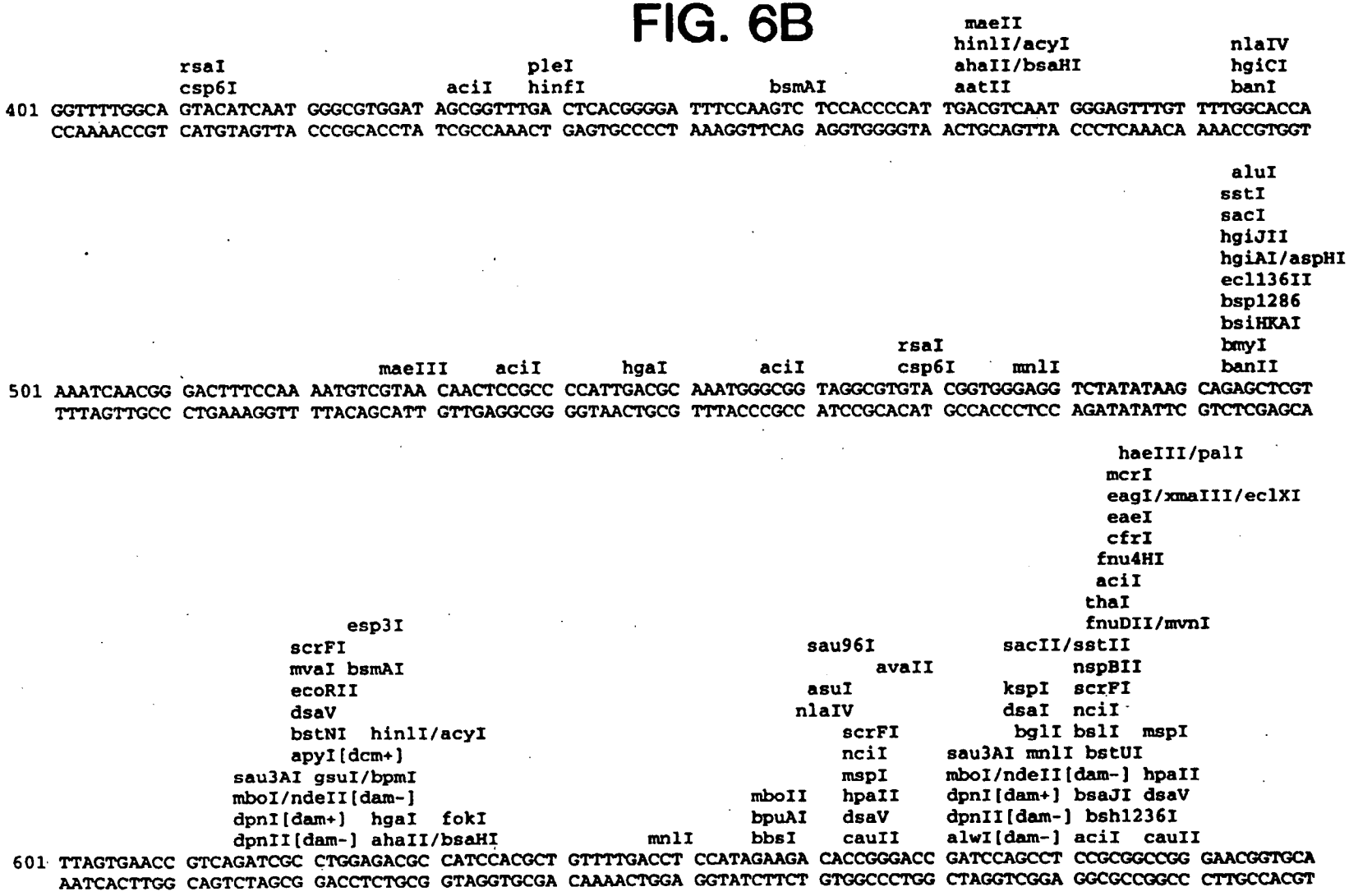

maeII
hinlI/acyI
nlaIV
rsaI
pleI
ahaII/bsaHI
hgiCI
csp6I
aciI
hinfI
bsmAI
aatII
banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT
aluI
sstI
sacI
hgiJII
hgiAI/aspHI
ecl136II
bsp1286
bsiHKAI
rsaI
bmyI
maeIII
aciI
hgaI
aciI
csp6I
mnlI
banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA
haeIII/palI
mcrI
eagI/xmaIII/eclXI
eaeI
cfrI
fnu4HI
aciI
thaI
fnuDII/mvnI
esp3I
scrFI
sau96I
sacII/sstII
mvaI bsmAI
avaII
nspBII
ecoRII
asuI
kspI scrFI
dsaV
nlaIV
dsaI nciI
bstNI hinlI/acyI
scrFI
bglI bslI mspI
apyI[dcm+]
nciI
sau3AI mnlI bstUI
sau3AI gsuI/bpmI
mspI
mboI/ndeII[dam-] hpaII
mboI/ndeII[dam-]
mboII
hpaII
dpnI[dam+] bsaJI dsaV
dpnI[dam+] hgaI fokI
bpuAI
dsaV
dpnII[dam-] bsh1236I
dpnII[dam-] ahaII/bsaHI
mnlI
bbsI
cauII
alwI[dam-] aciI cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT

FIG. 6C

```
                 tfiI
             aciI
            thaI hinfI                                                           fnu4HI
            fnuDII/mvnI                         aciI                             bbvI
            bstUI                            rsaI                              nspBII           taqI
            bsh1236I                         csp6I   scfI           mnlI           aciI       nlaIII
  701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
      AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                   thaI
                                                                                   fnuDII/mvnI
                                                                                   bstUI
                                                                                   bsh1236I
                                                                                 mluI
                                                                      bsrBI      aflIII     rsaI
                       pflMI                        bsmAI             aciI     xmnI         csp6I
      sfaNI            bslI                         bsaI             mnlI   ddeI asp700    scaI
  801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
      CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                             scrFI
                                                             mvaI
                                                             ecoRII
                                                             dsaV
           eco57I                                            bstNI                   tfiI       tru9I         tru9I
          mboII                tfiI                          apyI[dcm+]            mboII  taqI  mseI          mseI
         earI/ksp632I          hinfI                                                                          
        mnlI                 alwNI     hphI                sexAI            ddeI     hinfI     ahaIII/draI  aseI/asnI/vspI
  901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
      TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                             aluI
                                            sstI
                                            sacI
                                            hgiJII
                                            hgiAI/aspHI
                                            ecl136II
                                            bsp1286
                                            bsiHKAI
                                            bmyI                                     tru9I              mspI
                                            banII                           sfaNI    mseI               hpaII
             ddeI                    bslI  mnlI                 bstXI      fokI     aflII/bfrI        bsaWI
 1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
      TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 6D

```
                                                                               haeIII/palI
                                                                              haeI
                                                       scrFI          scrFI                        nlaIII
                                                       mvaI                 mvaI                            sau3AI
                                                       ecoRII         ecoRII                   mboI/ndeII[dam-]
                                                       dsaV   tfiI    dsaV    pleI             dpnI[dam+]
                                                       bstNI     nlaIII     bstNI     ddeI                  dpnII[dam-]
             accI  nlaIII             mnlI             apyI[dcm+]   hinfI   apyI[dcm+]    hinfI     maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                          mnlI
                                                                                     hinlI/acyI         scrFI
                                                                                     ahaII/bsaHI        mvaI
                                                                                  scrFI          ecoRII
                                                                                  mvaI    ecoNI         dsaV
                                                                                  ecoRII      sau96I
                                                                                  dsaV        avaII
                         maeII                                                    bstNI   bslI      asuI    mnlI
                       aflIII                                                     apyI[dcm+]     mnlI   bstNI
         apoI       maeIII                                       mnlI          bsaJI  hgaI     ddeI     apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                         ppu10I
                                                             sfaNI                                       nsiI/avaIII
         sfaNI                 accI     mboII            mboII                           mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                         fnu4HI
        nlaIII                                           aciI
       styI                                             thaI
       ncoI                                             fnuDII/mvnI tru9I
       dsaI                          styI               bstUI       mseI                                      maeIII
       bsaJI                         bsaJI              bsh1236I   aseI/asnI/vspI                            hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 6E

```
                                                                                                    haeIII/palI
                                                                                                   eaeI
                                                        scrFI                                   cfrI
                                                        mvaI                                         mspI
                                                        ecoRII                                 hpaII
                                                        dsaV                                  scrFI
                                                        bstNI                                        nciI
                                                        apyI[dcm+]      mnlI              ecoRI      dsaV
                                                      gsuI/bpmI         bsaJI       taqI  apoI       cauII
       scfI        fokI                            maeIII     hincII/hindII       claI/bsp106   bsaJI         aluI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATAGCTAACT TAAGGGGCCG GTATCGACAG

                                                          gsuI/bpmI
                                                         scrFI
                                                         mvaI
                                                         ecoRII
                                                         dsaV                                               mnlI
              mnlI                              nspBII   bstNI                                              ecoNI
            haeIII/palI                     fnu4HI    hgiAI/aspHI                          ddeI               sau96I
           sau96I                        fnu4HI aciI  bsp1286                             mnlI                nlaIV
           asuI                          bbvI  fnu4HI bsiHKAI    aluI                     eco81I              avaII
        nlaIII                      bspMI   bbvI      bmyI  apyI[dcm+]                    bsu36I/mstII/sauI   asuI  hphI
1601 TGGCATGGGC CTCTCCACCG TGCCTGACCT GCTGCTGCCG CTGGTGCTCC TGGAGCTGTT GGTGGGAATA TACCCCTCAG GGGTTATTGG ACTGGTCCCT
     ACCGTACCCG GAGAGGTGGC ACGGACTGGA CGACGACGGC GACCACGAGG ACCTCGACAA CCACCCTTAT ATGGGGAGTC CCCAATAACC TGACCAGGGA

        rmaI
        maeI
       styI
       bsaJI
       blnI            mboII                  styI                                         rsaI
       avrII           earI/ksp632I           bsaJI                mnlI         taqI       csp6I              nlaIV
1701 CACCTAGGGG ACAGGGAGAA GAGAGATAGT GTGTGTCCCC AAGGAAAATA TATCCACCCT CAAAATAATT CGATTTGCTG TACCAAGTGC CACAAAGGAA
     GTGGATCCCC TGTCCCTCTT CTCTCTATCA CACACAGGGG TTCCTTTTAT ATAGGTGGGA GTTTTATTAA GCTAAACGAC ATGGTTCACG GTGTTTCCTT
```

FIG. 6F

```
                                 scrFI
                                 nciI
                                 mspI
                                 hpaII
                                 dsaV
                                 cauII
                                xmaI/pspAI
                                smaI
                                scrFI
                                nciI
                                dsaV
                                cauII
                                bslI
                              sau96I
                              haeIII/palI
                              asuI
                           scrFI
                           mvaI bsaJI
                           ecoRII
                           dsaV                                   nlaIV
            rsaI           bstNI bsaJI         scfI                   fnu4HI                               alwNI
            csp6I          bslI avaI           pstI                   aciI          aciI              ddeI
           bsp1407I        apyI[dcm+]          bsgI                bsrBI       hphI    eco57I        mnlI
1801 CCTACTTGTA CAATGACTGT CCAGGCCCGG GGCAGGATAC GGACTGCAGG GAGTGTGAGA GCGGCTCCTT CACCGCTTCA GAAAACCACC TCAGACACTG
     GGATGAACAT GTTACTGACA GGTCCGGGCC CCGTCCTATG CCTGACGTCC CTCACACTCT CGCCGAGGAA GTGGCGAAGT CTTTTGGTGG AGTCTGTGAC

                                                                         scrFI
                                                      mboII              nciI
                                                     earI/ksp632I        mspI
          fnu4HI                                  sau3AI                 hpaII
         aluI                                     mboI/ndeII[dam-]       dsaV                  scfI              mspI
        pvuII                                     dpnI[dam+]             cauII                 pstI              hpaII
        nspBII                                    dpnII[dam-]         sau96I                   bsgI           rsaI
      ddeI                                       bstYI/xhoII          avaII                   fnu4HI          csp6I
     mnlI bbvI                                   bglII                asuI     draIII         bbvI   mboII   bsrI cfr10I
1901 CCTCAGCTGC TCCAAATGCC GAAAGGAAAT GGGTCAGGTG GAGATCTCTT CTTGCACAGT GGACCGGGAC ACCGTGTGTG GCTGCAGGAA GAACCAGTAC
     GGAGTCGACG AGGTTTACGG CTTTCCTTTA CCCAGTCCAC CTCTAGAGAA GAACGTGTCA CCTGGCCCTG TGGCACACAC CGACGTCCTT CTTGGTCATG
```

FIG. 6G

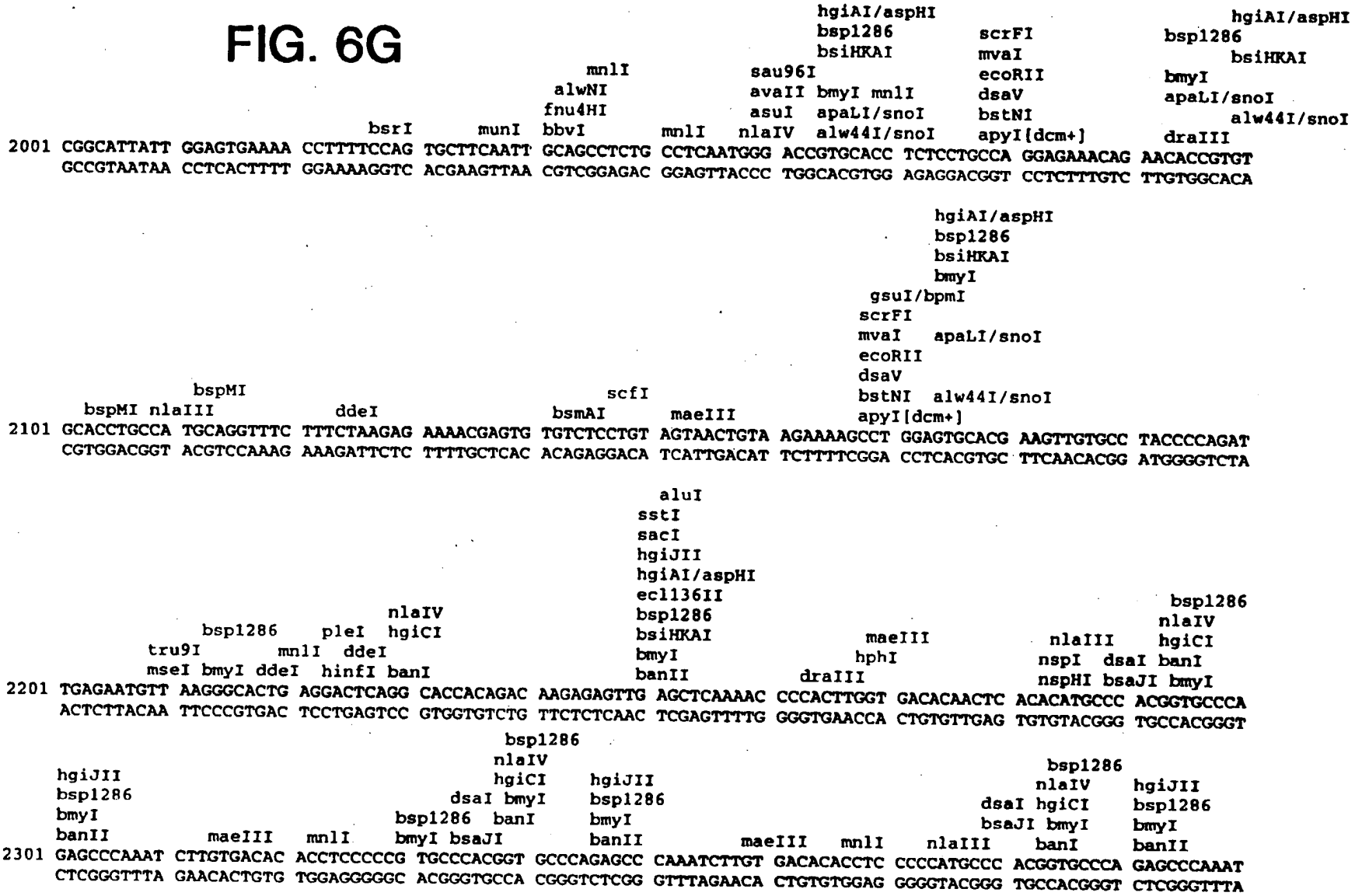


FIG. 6H

```
                                                    eam1105I
                                                   sau96I
                                             scrFI
                             bsp1286         mvaI  avaII
                            nlaIV            ecoRII
                            hgiCI            dsaV
                         dsaI bmyI           bstNI asuI    mboII mboII
                         bsaJI               bsaJI mnlI   bpuAI earI/ksp632I     styI
       maeIII  mnlI  nlaIII  banI  alwNI     apyI[dcm+]   bbsI mnlI              bsaJI
2401 CTTGTGACAC ACCTCCCCCA TGCCCACGGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ATACCCTTAT
     GAACACTGTG TGGAGGGGGT ACGGGTGCCA CGGGTCGTGG ACTTGAGGAC CCTCCTGGCA GTCAGAAGGA GAAGGGGGGT TTTGGGTTCC TATGGGAATA

            sau96I
            nlaIV
            avaII
            asuI        maeII
          mspI         pmlI
          hpaII        eco72I                                    sau96I
         scrFI   mnlI  bsaAI                          drdI       avaII              maeII
         nciI   ddeI maeIII                         mboII    mnlI                  bsaAI
         dsaV   eco81I bbrPI                        bpuAI  bsaJI  bsrI             rsaI
         cauII  bsu36I/mstII/sauI      maeII        bbsI  avaI   asuI              csp6I         mnlI
2501 GATTTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCACG AAGACCCCGA GGTCCAGTTC AAGTGGTACG TGGACGGCGT GGAGGTGCAT
     CTAAAGGGCC TGGGGACTCC AGTGCACGCA CCACCACCTG CACTCGGTGC TTCTGGGGCT CCAGGTCAAG TTCACCATGC ACCTGCCGCA CCTCCACGTA

                   aciI
                  thaI
                  fnuDII/mvnI
                  bstUI
                  bsh1236I
                 sacII/sstII
                 nspBII                                                    scrFI
                 kspI                                                      mvaI bsrI
                 dsaI                                                      ecoRII
                 bsaJI                                                     dsaV
                 aciI                                  mnlI     ecoNI      bstNI                rsaI
                fnu4HI mnlI           maeII          hgaI   hphI  bslI      apyI[dcm+]           csp6I
2601 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA CGTTCCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC GGCAAGGAGT
     TTACGGTTCT GTTTCGGCGC CCTCCTCGTC AAGTTGTCGT GCAAGGCACA CCAGTCGCAG GAGTGGCAGG ACGTGGTCCT GACCGACTTG CCGTTCCTCA
```

FIG. 6I

```
                 bsmAI                                                                       rsaI
                                                                                             csp6I
                bsaI           mnlI           taqI                                avaI       bsp1407I    bslI
2701 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAA CCAAAGGACA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
     TGTTCACGTT CCAGAGGTTG TTTCGGGAGG GTCGGGGGTA GCTCTTTTGG TAGAGGTTTT GGTTTCCTGT CGGGGCTCTT GGTGTCCACA TGTGGGACGG

           scrFI
           nciI
           mspI
           hpaII
           dsaV
           cauII
          xmaI/pspAI
          smaI
          scrFI                   scrFI         scrFI
          nciI                    mvaI                mvaI
          dsaV                    ecoRII        ecoRII
          cauII                   dsaV          dsaV                                            aciI
          bsaJI                   bstNI               bstNI                               dsaI             nspBII
       fokI                       apyI[dcm+]          apyI[dcm+]                          bslI            fnu4HI fnu4HI
     bslI avaI  mnlI             sexAI           bspMI                                    bsaJI           bbvI   bbvI
2801 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG
     GGGTAGGGCC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT CTCGTCGCCC

                                                                                       dsaI
        mspI                                  pleI                mnlI            hphI                   fnu4HI
        hpaII                     mnlI  nlaIII hinfI     nlaIV   mboII   scfI     aluI  bsaJI        bspMI   bbvI
2901 CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
     GTCGGCCTCT TGTTGATGTT GTGGTGCGGA GGGTACGACC TGAGGCTGCC GAGGAAGAAG GAGATGTCGT TCGAGTGGCA CCTGTTCTCG TCCACCGTCG

                                                                                                              scrFI
                                                                                                              nciI
                                                                                                              mspI
                                                                                                              hpaII
                                                                                                              dsaV
                                                                                        scrFI                 cauII
                                                                                        nciI                  bsaJI
                                                                                        mspI                haeIII/palI
                                    nlaIII                                              hpaII              mcrI
                                ppu10I                            sapI                  dsaV               eagI/xmaIII/eclXI
         xmnI mboII             nsiI/avaIII                       mboII  mnlI       bsmAI                  eaeI
         asp700      nlaIII     sfaNI   mnlI          aciI        earI/ksp632I bslI     cauII              cfrI
3001 AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC TTCACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGAG TGCGACGGCC
     TCCCCTTGTA GAAGAGTACG AGGCACTACG TACTCCGAGA CGTGTTGGCG AAGTGCGTCT TCTCGGAGAG GGACAGAGGC CCATTTACTC ACGCTGCCGG
```

FIG. 6J

```
                rmaI
              mnlI
           sau3AI
           mboI/ndeII[dam-]                    styI
           dpnI[dam+]                        aciI
           dpnII[dam-]  taqI                fnu4HI   sau96I
           alwI[dam-]  salI   scfI         sfiI ncoI haeIII/palI
          nlaIV  maeI  hincII/hindII       haeIII/palI
          bstYI/xhoII  accI   pstI        eaeI  dsaI asuI              aluI
          bamHI xbaI pleI     bsgI   aluI cfrI  bsaJI                fnu4HI
          alwI[dam-] hinfI bspMI    hindIII bglI nlaIII               bbvI          maeIII            sfaNI   apoI
3101 GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT
     CCCCTAGGAG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC GTAGTGTTTA

                                                                                                    sau3AI
                                                                                                    mboI/ndeII[dam-]
                                                                                                    dpnI[dam+]
                                                                                                    dpnII[dam-]
                                                                                                   pvuI/bspCI
                                                                                                   mcrI
                                                                                                  taqI[dam-]   tru9I
                                                                                                 claI/bsp106[dam-]
                                                                                                sau3AI         mseI
                                                                                                mboI/ndeII[dam-]
                                                                                                dpnI[dam+] xmnI        hinPI
                                                                                                dpnII[dam-]  aseI/asnI/vspI
                                    rmaI
                                bsmI  maeI                                                nlaIII  alwI[dam-]  asp700      hhaI/cfoI
3201 TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC
     AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA GCTAGCCCTT AATTAAGCCG

                                                rsaI
                                                csp6I
           haeI                                nlaIV
         styI                                  kpnI
         ncoI                                  hgiCI
        dsaI haeIII/palI                       banI                      aluI
     fnu4HI nlaIII                             asp718     mnlI          pvuII
     bbvI  bsaJI             mnlI      mnlI    acc65I   ddeI aciI       nspBII
3301 GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG
     CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT ACACACAGTC AATCCCACAC
```

FIG. 6K

```
             nlaIV
         scrFI                                                          scrFI          scrFI                   nlaIV
         mvaI                                     ppu10I                       mvaI                      mvaI
         ecoRII                                 nsiI/avaIII             ecoRII         ecoRII
         dsaV                                  nlaIII                   dsaV           dsaV
         bstNI                                    sphI                         bstNI                     bstNI
         apyI[dcm+]                               nspI sfaNI                   apyI[dcm+]                apyI[dcm+]
        bsaJI                                     nspHI                       sexAI                     bsaJI
3401 GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG
     CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG GGTCCGAGGG GTCGTCCGTC

               sfaNI
             ppu10I
             nsiI/avaIII
            nlaIII
           sphI
           nspI                                                   aciI
           nspHI                            aciI        aciI  fokI               aciI bsrI  aciI           bslI
                                                                                                          aciI
3501 AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG
     TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG GGTCAAGGCG GGTAAGAGGC

                                                                                                          rmaI
                                                                                                         styI
                                                                                                         bsaJI
                                                 fnu4HI                                                  blnI
   nlaIII                                       sfiI mnlI                                                avrII
  styI                                          haeIII/palI                                            haeIII/palI
  ncoI                                      bsaJI bglI         ddeI                                   stuI
  dsaI                                    haeIII/palI bsaJI  mnlI  aluI                    mnlI       haeI
  bsaJI                                 mnlI  mnlI aciI    haeIII/palI                 mnlI         mnlI maeI
3601 CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG
     GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC TCCGAAAAAA CCTCCGGATC

                                                                               scrFI
                                                                               mvaI
                                                                               ecoRII
               tru9I                  haeIII/palI                              dsaV
              hpaI                   eaeI                                      bstNI
              hincII/hindII          cfrI                        bsrI          apyI[dcm+]       tru9I       fnu4HI
          aluI mseI  aluI           bsrI               maeII maeIII           bsaJI   maeIII    mseI        bbvI
3701 GCTTTTGCAA AAAGCTGTTA ACAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG GGAAAACCCT GGCGTTACCC AACTTAATCG CCTTGCAGCA
     CGAAAACGTT TTTCGACAAT TGTCGAACCG TGACCGGCAG CAAAATGTTG CAGCACTGAC CCTTTTGGGA CCGCAATGGG TTGAATTAGC GGAACGTCGT
```

FIG. 6L

```
                                                                                    hinPI
                                                                                     hhaI/cfoI
                                                 sau3AI                             nlaIV
                                       sau96I    mboI/ndeII[dam-]                   narI
                                       haeIII/palI                                  kasI
                                       asuI      dpnI[dam+]                         hinlI/acyI
                                                 dpnII[dam-]                        hgiCI
                   aluI              mnlI                                           haeII     aciI
                  pvuII           mboII   aciI  pvuI/bspCI                          banI    sfaNI
      fokI        nspBII          earI/ksp632I  mcrI                      bglI      ahaII/bsaHI
3801 CATCCCCCCT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGTA GCCTGAATGG CGAATGGCGC CTGATGCGGT
     GTAGGGGGGA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCAT CGGACTTACC GCTTACCGCG GACTACGCCA

                                                                                       aciI
                                                                                     fnu4HI
                                                               hinPI                 aciI
                                                              thaI                  thaI
                                                              fnuDII/mvnI           fnuDII/mvnI
                                                              bstUI scfI    hinPI   bstUI
                                                              bsh1236I      hhaI/cfoI hinPI
                                                           rsaI hhaI/cfoI fnu4HI  tru9I bsh1236I
                  sfaNI     aciI       aciI    maeII       csp6I    bslI  aciI    mseI hhaI/cfoI
3901 ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAT ACGTCAAAGC AACCATAGTA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
     TAAAAGAGGA ATGCGTAGAC ACGCCATAAA GTGTGGCGTA TGCAGTTTCG TTGGTATCAT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

         fnu4HI
        hinPI
        hhaI/cfoI                          hinPI
       thaI                                hhaI/cfoI
       fnuDII/mvnI                       rmaI                                               mspI
       bstUI                          hinPI  haeII                                          hpaII
       bsh1236I    aciI               hhaI/cfoI   bsrBI                                    naeI
     maeIII bbvI  maeIII             haeII maeI   aciI          mboII               maeII  cfr10I
4001 GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
     CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

                  nlaIV
                  hgiJII
                  bsp1286                                  nlaIV                               maeII    haeIII/palI
                  bmyI                                     hgiCI   taqI                        draIII   sau96I
      aluI        banII          nlaIV                     banI mnlI                  hphI     bsaAI    asuI
4101 AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTTGGGT GATGGTTCAC GTAGTGGGCC
     TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA ACTAAACCCA CTACCAAGTG CATCACCCGG
```

FIG. 6M

```
                                       maeII pleI           tru9I    pleI                                bslI
                                      drdI   hinfI  maeII   mseI     hinfI           bsrI               bslI  avaI
4201 ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG
     TAGCGGGACT ATCTGCCAAA AAGCGGGAAA CTGCAACCTC AGGTGCAAGA AATTATCACC TGAGAACAAG GTTTGACCTT GTTGTGAGTT GGGATAGAGC

                                                                                             thaI
                                                                                             fnuDII/mvnI
                                                         tru9I                          tru9I   apoI tru9I      tru9I
                                                         mseI           tru9I           mseI bstUI   mseI       mseI
                                              haeIII/palI          aluI    mseI    apoI     bsh1236I        sspI
4301 GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT AAAAAATGAG CTGATTTAAC AAAAATTTAA CGCGAATTTT AACAAAATAT
     CCGATAAGAA AACTAAATAT TCCCTAAAAC GGCTAAAGCC GGATAACCAA TTTTTTACTC GACTAAATTG TTTTTAAATT GCGCTTAAAA TTGTTTTATA

                         hgiAI/aspHI
                         bsp1286
                         bsiHKAI                                                                               hinPI
                         bmyI    ddeI                    aciI                               maeIII         fnu4HI
       maeII             apaLI/snoI   rsaI              fnu4HI    tru9I                   maeII bsrI    nlaIII hhaI/cfoI
      psp1406I           alw44I/snoI  csp6I          sfaNI        mseI        aciI       bsaAI tth111I/aspI bbvI
4401 TAACGTTTAC AATTTTATGG TGCACTCTCA GTACAATCTG CTCTGATGCC GCATAGTTAA GCCAACTCCG CTATCGCTAC GTGACTGGGT CATGGCTGCG
     ATTGCAAATG TTAAAATACC ACGTGAGAGT CATGTTAGAC GAGACTACGG CGTATCAATT CGGTTGAGGC GATAGCGATG CACTGACCCA GTACCGACGC

                                                                                                  scrFI
                                  hinPI                         sfaNI                             nciI
                                  hhaI/cfoI                  mspI                                 mspI
                                 thaI                        hpaII                                hpaII     nspI
                                 fnuDII/mvnI                scrFI                                 dsaV      nspHI
                                 bstUI                      nciI                             esp3I      fnu4HI
                         nspBII  bsh1236I                   dsaV fokI                  maeIII bsmAI     bbvI
              aciI       aciI  hgaI       drdI              cauII   aciI           aluI    bslI   cauII  aluI nlaIII
4501 CCCCGACACC CGCCAACACC CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT
     GGGGCTGTGG GCGGTTGTGG GCGACTGCGC GGGACTGCCC GAACAGACGA GGGCCGTAGG CGAATGTCTG TTCGACACTG GCAGAGGCCC TCGACGTACA

                                       thaI
                                       fnuDII/mvnI
                                       bstUI
                                       bsh1236I
                                      hinPI
                                      hhaI/cfoI                 mnlI
                                    thaI                     haeIII/palI
                                    fnuDII/mvnI         mboII      sau96I                                  nlaIII
                                    bstUI mnlI          bpuAI      asuI                            tru9I  rcaI
         mnlI    hphI      hphI     bsh1236I            bbsI      eco0109I/draII                   mseI   bspHI
4601 GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA GTATTCTTGA AGACGAAAGG GCCTCGTGAT ACGCCTATTT TTATAGGTTA ATGTCATGAT
     CAGTCTCCAA AAGTGGCAGT AGTGGCTTTG CGCGCTCCGT CATAAGAACT TCTGCTTTCC CGGAGCACTA TGCGGATAAA AATATCCAAT TACAGTACTA
```

FIG. 6N

```
                                                           nlaIV
                                                         aciI
                                                        thaI
                                                        fnuDII/mvnI
                  hinlI/acyI                            bstUI                                                     rcaI
                  ahaII/bsaHI                           bsh1236I                                                  bspHI
                  aatII                                hinPI                                                   bsrBI
              ddeI maeII                               hhaI/cfoI                                               aciI nlaIII
4701 AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
     TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG

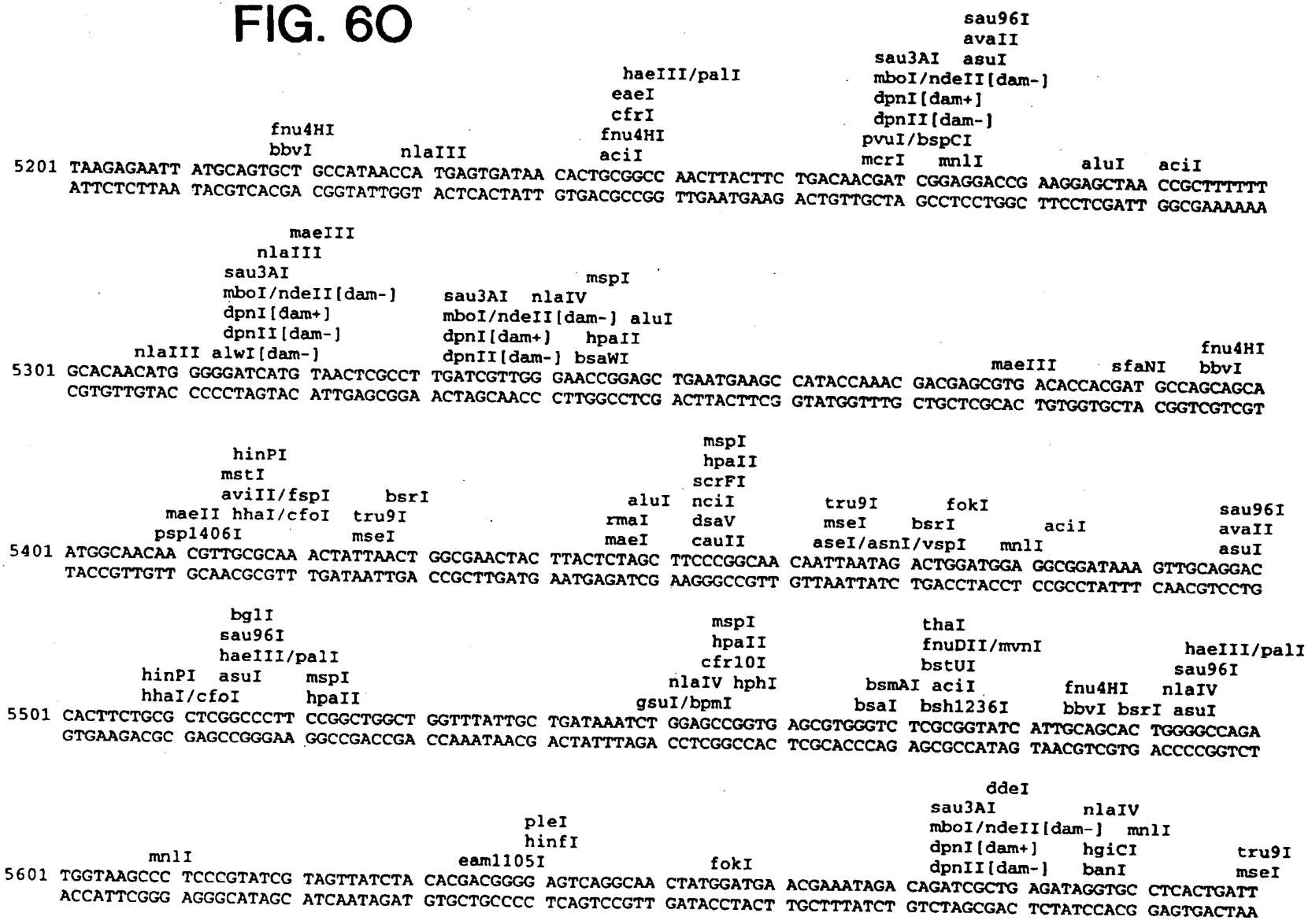
                                                      mboII                                                    fnu4HI
       bsmAI                              sspI        earI/ksp632I                                             aciI
4801 ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
     TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA

                                                                         hgiAI/aspHI
                                                                         bsp1286
                                                               sau3AI    bsiHKAI                                sau3AI
                                                               mboI/ndeII[dam-]                                 mboI/ndeII[dam-]
                                                               dpnI[dam+] bmyI                                  dpnI[dam+]
                                                               dpnII[dam-]                                     bstYI/xhoII
                                                           eco57I        apaLI/snoI                         bsrI dpnII[dam-]
                         hphI              hphI          sfaNI  mboII[dam-]  alw44I/snoI  maeIII taqI        alwI[dam-]
4901 TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
     AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG ACCTAGAGTT

                                                                                      aciI
                sau3AI                                                               thaI
                mboI/ndeII[dam-]             maeII                                   fnuDII/mvnI
                dpnI[dam+]                  psp1406I           hgiAI/aspHI           bstUI
                dpnII[dam-]                 xmnI               bsp1286 tru9I         bsh1236I            hinlI/acyI
          aciI  alwI[dam-]                  asp700             bsiHKAI mseI          hinPI               hgaI
     nspBII  bstYI/xhoII                  mboII                bmyI   ahaIII/draI    hhaI/cfoI           ahaII/bsaHI
5001 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC
     GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCACTACTG

      scrFI
      nciI
      mspI
      hpaII                                                      rsaI
      dsaV                     aciI                              csp6I   bsrI
      cauII     bcgI  mcrI  fnu4HI          ddeI               scaI  hphI  maeIII      sfaNI     fokI    nlaIII
5101 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
     CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA CCGTACTGTC
```

FIG. 6O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avaII
sau3AI asuI
haeIII/palI
mboI/ndeII[dam-]
eaeI
dpnI[dam+]
cfrI
dpnII[dam-]
fnu4HI
pvuI/bspCI
bbvI
nlaIII
aciI
mcrI mnlI
aluI aciI
5201 TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT
ATTCTCTTAA TACGTCACGA CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA GCCTCCTGGC TTCCTCGATT GGCGAAAAAA
maeIII
nlaIII
sau3AI
mspI
mboI/ndeII[dam-]
sau3AI nlaIV
dpnI[dam+]
mboI/ndeII[dam-] aluI
dpnII[dam-]
dpnI[dam+] hpaII
nlaIII alwI[dam-]
dpnII[dam-] bsaWI
fnu4HI
maeIII sfaNI bbvI
5301 GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCAGCAGCA
CGTGTTGTAC CCCCTAGTAC ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC TGTGGTGCTA CGGTCGTCGT
hinPI
mspI
hpaII
mstI
scrFI
aviII/fspI bsrI
aluI nciI tru9I fokI
sau96I
maeII hhaI/cfoI tru9I
rmaI dsaV mseI bsrI aciI
avaII
psp1406I mseI
maeI cauII aseI/asnI/vspI mnlI
asuI
5401 ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC
TACCGTTGTT GCAACGCGTT TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC TGACCTACCT CCGCCTATTT CAACGTCCTG
bglI
mspI
thaI
sau96I
hpaII
fnuDII/mvnI
haeIII/palI
haeIII/palI
cfr10I
bstUI
sau96I
hinPI asuI mspI
nlaIV hphI
bsmAI aciI fnu4HI nlaIV
hhaI/cfoI hpaII
gsuI/bpmI
bsaI bsh1236I bbvI bsrI asuI
5501 CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA
GTGAAGACGC GAGCCGGGAA GGCCGACCGA CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG TAACGTCGTG ACCCCGGTCT
ddeI
sau3AI nlaIV
pleI
mboI/ndeII[dam-] mnlI
hinfI
dpnI[dam+] hgiCI tru9I
mnlI
eam1105I fokI
dpnII[dam-] banI mseI
5601 TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
ACCATTCGGG AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT GTCTAGCGAC TCTATCCACG GAGTGACTAA

FIG. 6P

```
                                                                                             rmaI     sau3AI
                                                                                         sau3AI hphI  mboI/ndeII(dam-)
                                                                                         mboI/ndeII(dam-)
                                                                                         dpnI(dam+)   dpnI(dam+)
                                                                                         dpnII(dam-)  dpnII(dam-)
                                                             tru9I              ahaIII/draI  maeI     alwI(dam-)
                                                             mseI       tru9I tru9I bstYI/xhoII  bstYI/xhoII
             maeIII                                         ahaIII/draI     mseI  mseI  alwI(dam-) mboII(dam-)
5701 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAGGAT CTAGGTGAAG ATCCTTTTTG
     TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC

                                                                                       sau3AI
                                                                                       mboI/ndeII(dam-)
                                                                                       dpnI(dam+)  sau3AI
                                                                                       dpnII(dam-) mboI/ndeII(dam-)
                                                                                      bstYI/xhoII  dpnI(dam+)
                                                                              sau3AI  alwI(dam-)   dpnII(dam-)
            nlaIII             maeII                                          mboI/ndeII(dam-)     alwI(dam-)
           rcaI              tru9I                      hgaI                  dpnI(dam+) mboII(dam-)
           bspHI             mseI                   ddeI                      dpnII(dam-)        bstYI/xhoII
5801 ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
     TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAAGA

                                                                        sau3AI
      thaI                                                              mboI/ndeII(dam-)
      fnuDII/mvnI                                                       dpnI(dam+)
      bstUI                                                             dpnII(dam-)
      bsh1236I                                                         alwI(dam-)
     hinPI      fnu4HI                              aciI               mspI                                   bsrI
     hhaI/cfoI  bbvI                      aciI    nspBII               hpaII     aluI                      maeIII  eco57I
5901 GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
     CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG

              hinPI                    rmaI                haeIII/palI
              hhaI/cfoI                maeI      bslI     haeI                 scfI    aciI       mnlI
6001 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
     AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG
```

FIG. 6Q

```
                                                         scrFI                                       aciI
                                                         nciI                                      nspBII
                           fnu4HI                        mspI                         mspI        fnu4HI
                  alwNI    bbvI                          hpaII                        hpaII       bbvI
                 bsrI  fnu4HI                            dsaV     pleI               bsaWI      hinPI mcrI
            maeIII     bbvI    bsrI                      cauII    hinfI           maeIII        hhaI/cfoI
6101 TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
     ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC

                  hgiAI/aspHI
                  bsp1286
                  bsiHKAI
                  bmyI                                                                          hinPI
                  apaLI/snoI                                                                    hhaI/cfoI
                  alw44I/snoI   aluI                          ddeI      scfI                   haeII
6201 AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA
     TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGTAACTC TTTCGCGGTG CGAAGGGCTT

                                                                             scrFI
                                                                             mvaI          scrFI
                                                                             ecoRII  mvaI
                            mspI                                             dsaV    ecoRII
                            hpaII                                            bstNI         dsaV
                            bslI   fnu4HI                   hinPI mnlI       bsaJI         bstNI
              aciI          bsaWI  aciI                     hhaI/cfoI   aluI apyI[dcm+]    apyI[dcm+]
6301 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
     CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC

                                                                                                  haeIII/palI
                                                                                                fnu4HI
                                                                                                aciI
                                                                                               thaI bslI
                                                                                               fnuDII/mvnI
                                   taqI                            nlaIV                       bstUI
                   mnlI drdI    hgaI           sfaNI             aciI                          bsh1236I       nlaIV
6401 TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
     AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA
```

FIG. 6R

```
   haeIII/palI
  haeI
scrFI
mvaI bslI
ecoRII                nlaIII
dsaV                  nspI
bstNI          haeIII/palI  nspHI                    tfiI                                                    bsrBI
apyI[dcm+]     haeI         aflIII                   hinfI                        aciI          aluI       aciI
6501 CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
     GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG ACTATGGCGA

                                                                                                  thaI
                                                                                                  fnuDII/mvnI
                                                                                                  bstUI
                                                                                                  bsh1236I
                                                                                                 hinPI
                                                                                                 hhaI/cfoI
                                                                                                thaI
                                                                                                fnuDII/mvnI
        fnu4HI              fnu4HI                        sapI  hinPI                            bstUI   haeIII/palI
        bbvI                bbvI pleI                     mboII hhaI/cfoI                       bsh1236I           tru9I
      aciI                  hinPI  hinfI                  earI/ksp632I              mnlI   bslI     eaeI  tfiI aseI/asnI/vspI
    fnu4HI       mcrI       hhaI/cfoI          mnlI   aciI    haeII          aciI       aciI     cfrI  hinfI mseI
6601 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
     GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA

                                                                                                  scrFI
                                                                                                  mvaI
                                                                                                  ecoRII
                                                                                                  dsaV
         aluI                                                  tru9I                              nlaIV bstNI
         pvuII                                          hinPI       mseI      maeIII              hgiCI apyI[dcm+]
         nspBII                      bsrI     aciI      hhaI/cfoI  aseI/asnI/vspI  mnlI         banI bsaJI
6701 AATCCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA
     TTAGGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT GGAGTGAGTA ATCCGTGGGG TCCGAAATGT

                                                                                                  tru9I
                                                                                                  mseI
                                                                                                 aseI/asnI/vspI
               mspI                          aciI                                               xmnI
               hpaII                        bsrBI                          aluI      nlaIII    asp700
6801 CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA
     GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAATT
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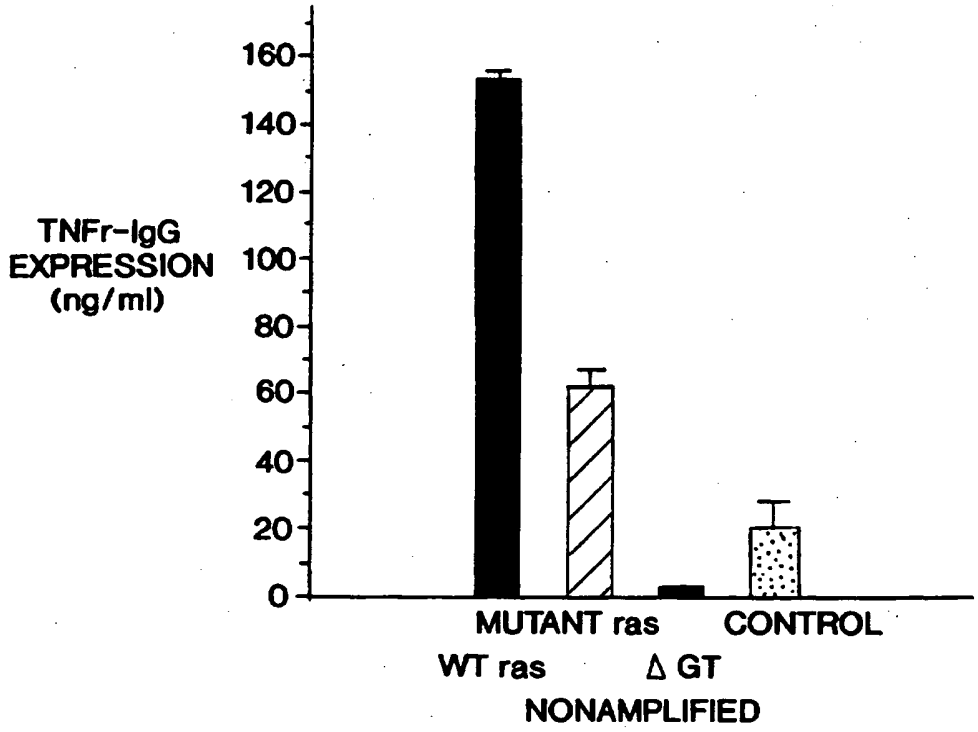
TNFr-IgG
EXPRESSION
(ng/ml)
160
140
120
100
80
60
40
20
0
MUTANT ras
CONTROL
WT ras
Δ GT
NONAMPLIFIED

FIG. 7A

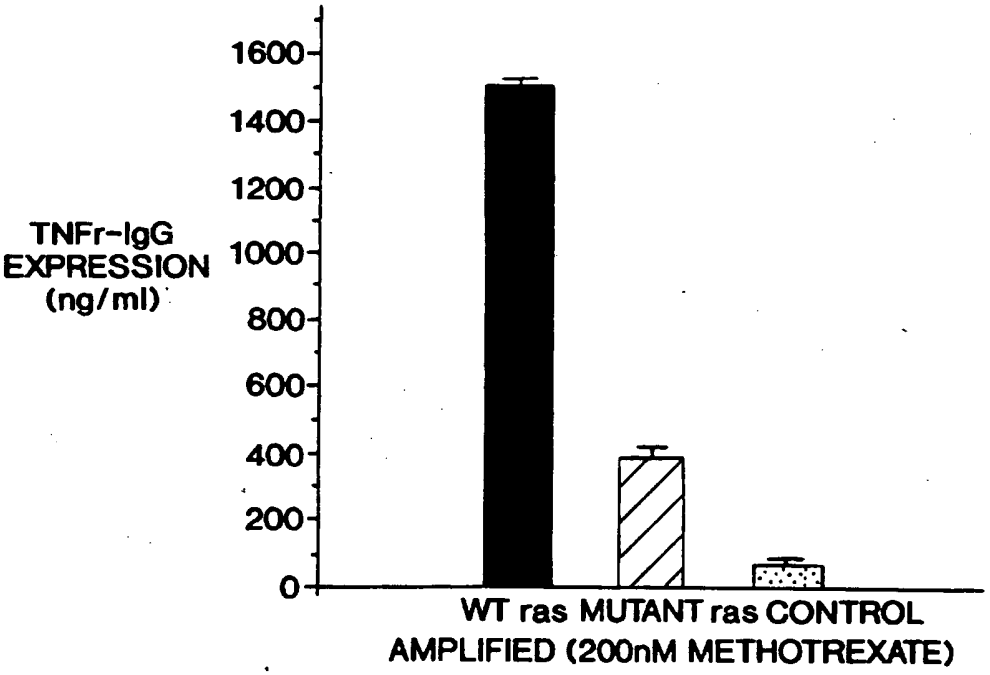
TNFr-IgG
EXPRESSION
(ng/ml)
1600
1400
1200
1000
800
600
400
200
0
WT ras MUTANT ras CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 7B

FIG. 8

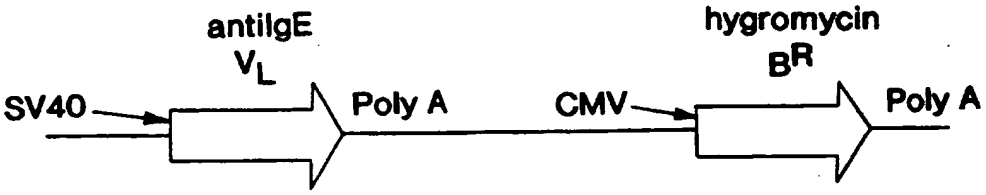
antiIgE
V_L
hygromycin
B^R
SV40
Poly A
CMV
Poly A

FIG. 11

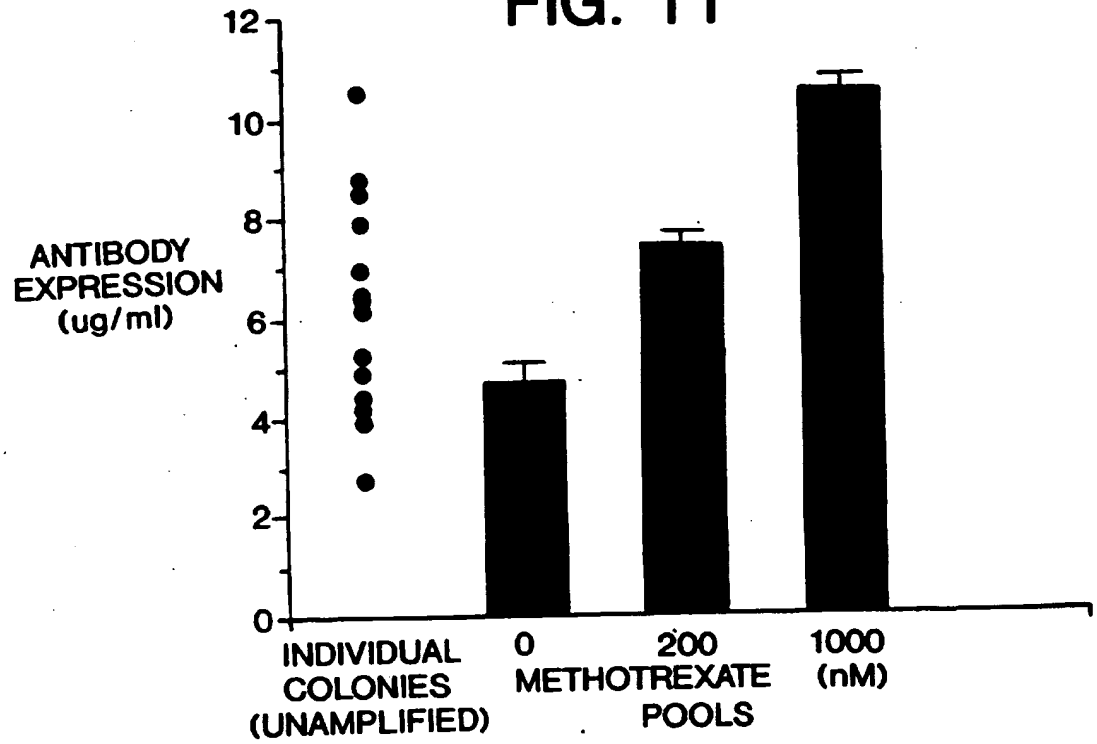
ANTIBODY EXPRESSION (ug/ml)
12
10
8
6
4
2
0
INDIVIDUAL COLONIES (UNAMPLIFIED)
0
200
1000
METHOTREXATE (nM)
POOLS

FIG. 9A

```
      aluI
     sstI
     sacI
     hgiJII
     hgiAI/aspHI
     ecl136II
     bsp1286
     bsiHKAI
     bmyI
     banII
    taqI
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                                                 aluI
                                     sau3AI pvuII
                                     mboI/ndeII[dam-]
                                     dpnI[dam+]
                                    pvuI/bspCI
                                 pleI dpnII[dam-]
                                 hinfI  taqI[dam-]
                              rmaI    mcrI    nspBII
                              maeI  taqI[dam-]

                                                                                        nlaIV
                                                                                      scrFI
                                                                                      mvaI
                                                                                      ecoRII
                                                                                      dsaV
                                                                                      bstNI
                                                                                      apyI[dcm+]
                                                                                     bsaJI

                                                                nlaIV
            sfaNI               scrFI        scrFI                            sfaNI
           ppu10I               mvaI               mvaI                                     ppu10I
           nsiI/avaIII          ecoRII       ecoRII                          nsiI/avaIII
          nlaIII                dsaV         dsaV                           nlaIII
         sphI                   bstNI              bstNI                                   sphI
         nspI                   apyI[dcm+]         apyI[dcm+]                              nspI
         nspHI                 sexAI              bsaJI                                    nspHI
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                              nlaIII
                                                                             styI
                                                                             ncoI
                                  aciI                                   bslI dsaI
            aciI          aciI   fokI          aciI bsrI  aciI           aciI bsaJI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA

                                                                        rmaI
                                                                       styI
            fnu4HI                                                     bsaJI                         haeIII/palI
            bglI                                                       blnI                         mcrI
           sfiI                                                        avrII               aluI     eagI/xmaIII/eclXI
           haeIII/palI                                               haeIII/palI          rmaI      eaeI
         mnlI   mnlI       ddeI                                     stuI                  maeI      cfrI
     haeIII/palI bsaJI  mnlI    aluI                  mnlI          haeI                 nheI       mspI
   mnlI bsaJI aciI    haeIII/palI                   mnlI           mnlI maeI             aluI       hpaII
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
```

FIG. 9B

```
                                tfiI
    scrFI                       hinfI
    ncil                     aciI
    mspI                    thaI
    hpaII                   fnuDII/mvnI                                                      fnu4HI
    dsaV                    bstUI                                     aciI                   bbvI
    cauII                   bsh1236I              maeII  rsaI                               nspBII
                                               maeIII  csp6I  scfI         mnlI            aciI       nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA

                                                                          haeIII/palI
                                                                           haeI
                                                                          scrFI
                                                                          mvaI     bsrBI
                                                                          ecoRII
                                                                          dsaV
                                                                          bstNI    aciI            rsaI
                                             pflMI               bsmAI    apyI[dcm+]        xmnI      csp6I
    taqI        sfaNI                        bslI                bsaI     bsaJI  mnlI  ddeI asp700    scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

                                                                         scrFI
                                                                         mvaI
                                                                         ecoRII
                 eco57I                                                  dsaV
                mboII                  tfiI                              bstNI                tfiI       tru9I
               earI/ksp632I            hinfI                             apyI[dcm+]           hinfI      mseI
              mnlI                   alwNI    hphI                      sexAI           ddeI mboII taqI  ahaIII/draI
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                            sstI
                                                            sacI
                                                            hgiJII
                                                            hgiAI/aspHI
                                                            ecl136II
                                                            bsp1286
                                                            bsiHKAI
        tru9I                                               bmyI                                   tru9I
        mseI          ddeI                                  banII                                 aflII/bfrI
       aseI/asnI/vspI                          bslI   mnlI aluI        bstXI      fokI sfaNI mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
```

FIG. 9C

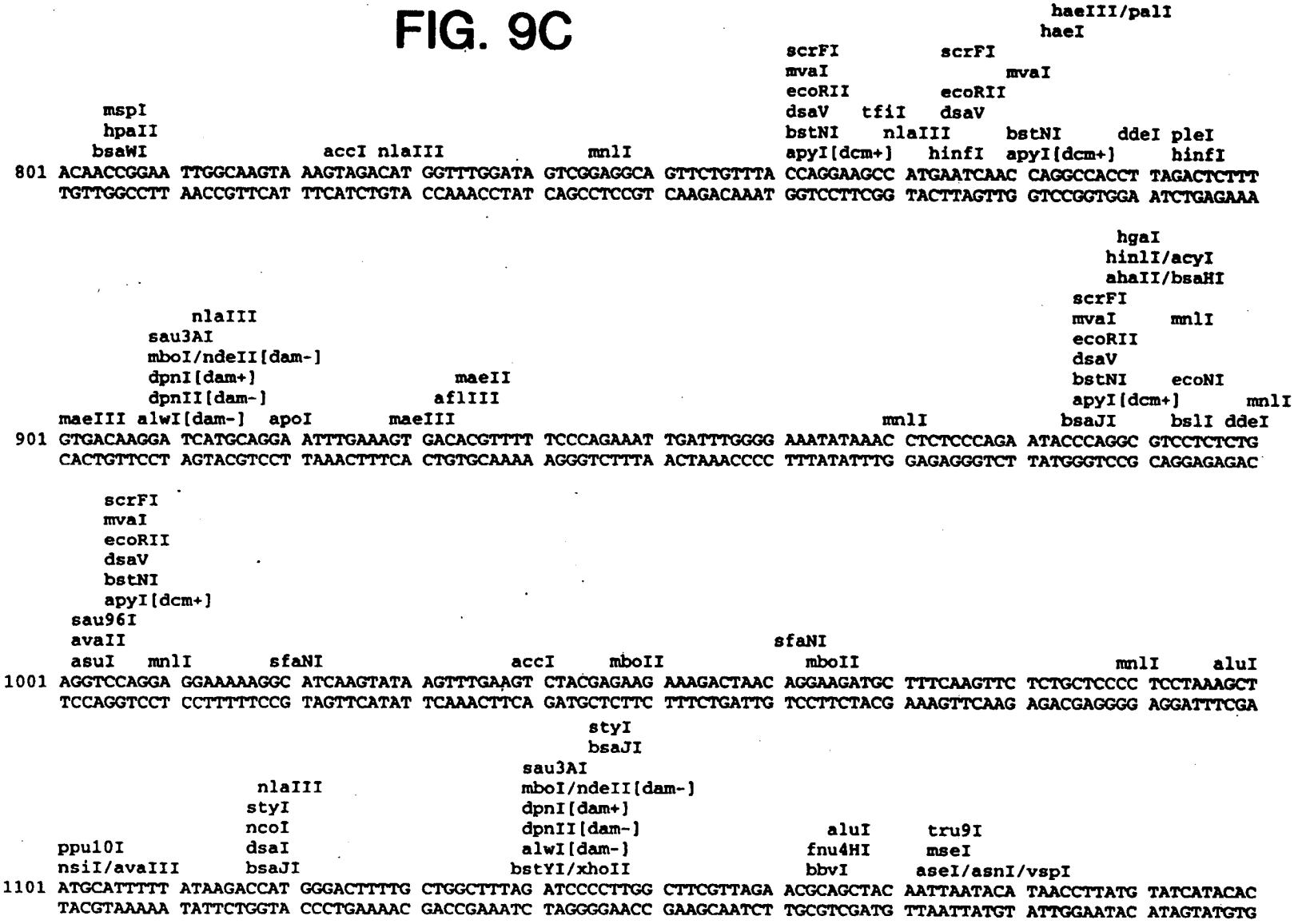

FIG. 9D

```
                                                                          sau96I
                                                                          avaII
                                                                          asuI
                                                                       scrFI
                                                                       mvaI
                                                                       ecoRII
                                                                       dsaV
                                                                       bstNI                                    ecoRI
               maeIII                                                  apyI[dcm+]       mnlI         taqI   apoI
              hphI    scfI         fokI                          bslI  bsaJI            bsaJI       claI/bsp106
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA

                                                                                                      scrFI
        nlaIII                                                                                        mvaI
       styI                                                                                           ecoRII
       pflMI                                                                                          dsaV
       ncoI                                                                                           bstNI fnu4HI
       dsaI                                                          rsaI            aluI             apyI[dcm+]
       bslI fokI                       rmaI                gsuI/bpmI                pvuII     pleI       haeI     bbvI
       bsaJI         nlaIII   fokI     maeI               bsrI    csp6I             nspBII    hinfI   aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTGGTGGAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGACCACCTC AGACCGCCAC CGGACCACGT
```

FIG. 9E

```
                                                                                                                  scrFI
                                                                                                                  nciI
                                                                                                                  mspI
                                                                                                                  hpaII
                                                                                                                  dsaV
                                                                                                                  cauII
                                                                                                                  bslI
                                                                                                                 xmaI/pspAI
                                                                                                                 smaI
                                                                                                                 scrFI
                                                                                                                 nciI
                                                                                                                 dsaV
                                                                                                                 cauII      scrFI
                                                                                                              sau96I        mvaI
                                                                                                    sau3AI    nlaIV         ecoRII
        hgiJII                                                                                      mboI/ndeII[dam-]        dsaV
        bsp1286                                                                  mspI          dpnI[dam+]  bslI        bstNI
        bmyI                                                               hpaII                    dpnII[dam-]  bsaJI     bslI
   scrFI                                                                      mroI         nlaIV       haeIII/palI    apyI[dcm+]
   mvaI banII                                                              bspMII          bstYI/xhoII    avaI      sau96I
   ecoRII                                                                  bspEI              bamHI  eco0109I/draII haeIII/palI
   dsaV                                                                       bsaWI           alwI[dam-] asuI         asuI
   bstNI                                                                    mnlI        aluI  bsrI alwI[dam-]  bsaJI   eco0109I/draII
   bsaJI                                        alwNI                    hphI  accIII
   apyI[dcm+]
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGTTTCTGGC TACTCCATCA CCTCCGGATA TAGCTGGAAC TGGATCCGTC AGGCCCCGGG TAAGGGCCTG
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCAAAGACCG ATGAGGTAGT GGAGGCCTAT ATCGACCTTG ACCTAGGCAG TCCGGGGCCC ATTCCCGGAC

                             sau3AI
                             mboI/ndeII[dam-]
                             dpnI[dam+]                                                      thaI
              maeII          dpnII[dam-]                                                     fnuDII/mvnI
         taqI snaBI         alwI[dam-]                hgaI      haeIII/palI                  bstUI  tfiI
        claI/bsp106       mspI                      rmaI       sau96I                        bsh1236I
     sfaNI     bsaAI      hpaII taqI[dam-]          maeI       asuI                        nruI     hinfI
1501 GAATGGGTTG CATCGATTAC GTATGCCGGA TCGACTAACT ATAACCCTAG CGTCAAGGGC CGTATCACTA TAAGTCGCGA CGATTCCAAA AACACATTCT
     CTTACCCAAC GTAGCTAATG CATACGGCCT AGCTGATTGA TATTGGGATC GCAGTTCCCG GCATAGTGAT ATTCAGCGCT GCTAAGGTTT TTGTGTAAGA
```

FIG. 9F

scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
hinPI
hhaI/cfoI
nlaIV
mnlI
xhoI
narI
paeR7I
kasI
aval
hinlI/acyI
hgiAI/aspHI
hgiCI
bsp1286
haeII
bsiHKAI fnu4HI
banI
bbvI taqI
ahaII/bsaHI
scfI
pstI
bsgI
bspMI
mnlI
ddeI drdI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTATTGT GCTCGAGGCA GCCACTATTT CGGCGCCTGG CACTTCGCCG TGTGGGGTCA
TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATAACA CGAGCTCCGT CGGTGATAAA GCCGCGGACC GTGAAGCGGC ACACCCCAGT
sau96I
haeIII/pal1
sau96I
nlaIV
hgiJII
bsp1286
bsp120I
apaI
mboII
dsaV
bstNI
hgiAI/aspHI
bsp1286
bsiHKAI
bsp1286 aci1 apyI[dcm+]
bmyI nspBII bsaJI
haeIII/pal1
asuI
fnu4HI
scrFI
mvaI
ecoRII
dsaV
bstNI
bsaJI
sau96I
haeIII/pal1
asuI
scrFI
mvaI
ecoRII
dsaV
bstNI hphI
apyI[dcm+] bsmAI haeIII/pal1 eco0109I/draII
bsaJI maeIII mnlI styI asuI bpuAI apyI[dcm+] mnlI
nlaIV bstEII esp3I bsaJI mnlI bsaJI bbsI bsaJI mnlI
1701 AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC

FIG. 9G

```
          scrFI                                            hinPI
          mvaI                                                  nlaIV
          ecoRII                                          narI
          ecoNI                                                 kasI                hgiAI/aspHI
          dsaV                    hphI                    hinlI/acyI          bsp1286      mspI
          bstNI                       mspI                      hgiCI               bsiHKAI    hpaII
          bslI                        hpaII                     haeII               bmyI       scrFI
          apyI[dcm+]                cfr10I                      banI          fnu4HI       nciI
      fnu4HI                        bsaWI  tth111I/aspI         ahaII/bsaHI   aciI  apaLI/snoI dsaV
      bbvI                    bslI  ageI maeIII            ddeI hhaI/cfoI nspBII alw44I/snoI cauII     scfI
1801 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA

                                                               nlaIV
      ddeI pleI                                       fnu4HI   hgiCI
     mnlI hinfI                                  mnlI bbvI     banI                    tfiI
     eco81I           mnlI  fnu4HI  maeIII bsp1286 rmaI       bsp1286                  hinfI                styI
     bsu36I/mstII/sauI ddeI bbvI   hphI    bmyI    maeI aluI  bmyI                 maeII                    bsaJI
1901 CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACTGTGCC CTCTAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC
     GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGACACGG GAGATCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG

                                                                                               eam1105I
                                                                                              sau96I
                                                                                      scrFI
                                                                                      mvaI    avaII
                                                                                      ecoRII
                         hgiJII                                                       dsaV
                         bsp1286                        nlaIII                        bstNI   asuI       mboII mboII
                         bmyI                          nspI          bsp1286          bsaJI  nlaIV      bpuAI earI/ksp632I
                         banII          maeIII         nspHI         bmyI  alwNI      apyI[dcm+]      bbsI mnlI
2001 CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC
     GTTCCACCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CGGGTGGCAC GGGTCGTGGA CTTGAGGACC CCCCTGGCAG TCAGAAGGAG
```

FIG. 9H

```
                                      sau96I
                                      nlaIV
                                      avaII
                                    mspI
                                   scrFI
                                   nciI
                             sau3AI hpaII
                             mboI/ndeII[dam-]
                             dpnI[dam+]                        nlaIII
                          nlaIII     dsaV         mnlI    nspI                                  drdI   mnlI
                         rcaI        cauII      ddeI maeIII                               mboII     ddeI
              styI       bspHI[dam-]   asuI     eco81I    nspHI                           bpuAI     eco81I
              bsaJI    mnlI  dpnII[dam-]        bsu36I/mstII/sauI           maeII         bbsI      bsu36I/mstII/sauI
2101 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA
     AAGGGGGGTT TTGGGTTCCT GTGGGAGTAC TAGAGGGCCT GGGGACTCCA GTGTACGCAC CACCACCTGC ACTCGGTGCT TCTGGGACTC CAGTTCAAGT

                                                aciI
                                               thaI
                                               fnuDII/mvnI
                                               bstUI
                                               bsh1236I
                                              sacII/sstII
                                              nspBII
                                              kspI
         maeII                                dsaI
        rsaI                                  bsaJI                               rsaI
        csp6I                                 aciI                                csp6I
     bsrI bsaAI          mnlI                 fnu4HI  mnlI      rsaI    maeII                        mnlI     econI
     bsrI bsaAI                                                 csp6I   bsaAI              hgaI    hphI    bslI
2201 ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT
     TGACCATGCA CCTGCCGCAC CTCCACGTAT TACGGTTCTG TTTCGGCGCC CTCCTCGTCA TGTTGTCGTG CATGGCACAC CAGTCGCAGG AGTGGCAGGA

       scrFI
       mvaI bsrI
       ecoRII
       dsaV
       bstNI                                bsmAI                                                        fnu4HI
       apyI[dcm+]             rsaI         bsaI             mnlI          taqI                           bbvI
                              csp6I
2301 GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
     CGTGGTCCTG ACCGACTTAC CGTTCCTCAT GTTCACGTTC CAGAGGTTGT TTCGGGAGGG TCGGGGGTAG CTCTTTTGGT AGAGGTTTCG GTTTCCCGTC
```

FIG. 9I

```
                                              scrFI
                                              nciI
                                              mspI
                                              hpaII
                                              dsaV
                                              cauII
                                             xmaI/pspAI
                                             smaI
                                             scrFI                                scrFI
                                             nciI                                 mvaI                scrFI
                                             dsaV                                 ecoRII      mvaI
                                             cauII                                dsaV        ecoRII
                     rsaI              fokI      mboII                            bstNI             dsaV
                     csp6I          bslI  bsaJI                                   apyI[dcm+]        bstNI
       avaI         bsp1407I       bslI   avaI earI/ksp632I                      sexAI        bspMI apyI[dcm+]
2401 CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG AAGAGATGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG
     GGGGCTCTTG GTGTCCACAT GTGGGACGGG GGTAGGGCCC TTCTCTACTG GTTCTTGGTC CAGTCGGACT GGACGGACCA GTTTCCGAAG ATAGGGTCGC

         dsaI                          mspI
         bslI                      fnu4HI                                           pleI                   mnlI
         bsaJI                     bbvI hpaII                            mnlI       hinfI     nlaIV   mboII   scfI    aluI
2501 ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA
     TGTAGCGGCA CCTCACCCTC TCGTTACCCG TCGGCCTCTT GTTGATGTTC TGGTGCGGAG GGCACGACCT GAGGCTGCCG AGGAAGAAGG AGATGTCGTT

                                               bpuAI
                                               bbsI                            nlaIII
        dsaI                                 maeII                          ppu10I                          sapI
        bsaJI                      fnu4HI   xmnI mboII                      nsiI/avaIII                     mboII  mnlI
     hphI           bspMI          bbvI     asp700         nlaIII          sfaNI    mnlI                    earI/ksp632I bslI
2601 GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
     CGAGTGGCAC CTGTTCTCGT CCACCGTCGT CCCCTTGCAG AAGAGTACGA GGCACTACGT ACTCCGAGAC GTGTTGGTGA TGTGCGTCTT CTCGGAGAGG

                                                                            styI
         scrFI                                taqI                        aciI
         nciI                                 salI                       fnu4HI   sau96I
         mspI                          rmaI  hincII/hindII               sfiI ncoI haeIII/palI
         hpaII                         maeI  accI    scfI                haeIII/palI
         dsaV                       sau96I  pleI     pstI               eaeI  dsaI asuI                   aluI
         cauII                      haeIII/palI      bsgI      aluI cfrI  bsaJI                         fnu4HI
     bsmAI                          asuI    hinfI bspMI     hindIII bglI nlaIII                         bbvI       maeIII
2701 CTGTCTCCGG GTAAATGAGT GCGACGGCCC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT
     GACAGAGGCC CATTTACTCA CGCTGCCGGG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA
```

FIG. 9J

```
                                                                                                          taqI[dam-]
                                                                                                         claI/bsp106[dam-]
                                                                                                        sau3AI
                                                                                                        mboI/ndeII[dam-]
                                                                                                        dpnI[dam+]
                                                          rmaI                                          dpnII[dam-]
        sfaNI    apoI                               bsmI  maeI                               nlaIII  alwI[dam-]
2801 AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT
     TTTCGTTATC GTAGTGTTTA AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA

          tru9I                                                         rsaI
       xmnI                                                             csp6I
      sau3AI  aseI/asnI/vspI    styI                                   nlaIV
      mboI/ndeII[dam-]          ncoI                                   kpnI
      dpnI[dam+]           fnu4HI  haeI                                hgiCI
      dpnII[dam-]          bbvI  dsaI haeIII/palI                      banI                         aluI
     pvuI/bspCI mseI     hinPI    bsaJI                                asp718    mnlI              pvuII
     mcrI   asp700       hhaI/cfoI nlaIII        mnlI     mnlI          acc65I   ddeI aciI         nspBII
2901 CGATCGGGAA TTAATTCGGC GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA
     GCTAGCCCTT AATTAAGCCG CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT

                                  nlaIV
                               scrFI                                                    scrFI
                               mvaI                                   ppu10I                 mvaI
                               ecoRII                              nsiI/avaIII          ecoRII
                               dsaV                               nlaIII                dsaV
                               bstNI                                 sphI                    bstNI
                               apyI[dcm+]                            nspI sfaNI              apyI[dcm+]
                              bsaJI                                  nspHI                  sexAI       bsaJI
3001 TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC
     ACACACAGTC AATCCCACAC CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG

       nlaIV                            sfaNI
     scrFI                       ppu10I
     mvaI                             nsiI/avaIII
     ecoRII                     nlaIII
     dsaV                      sphI
     bstNI                          nspI                                                 aciI
     apyI[dcm+]                     nspHI                        aciI        aciI   fokI         aciI
3101 CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC
     GGTCCGAGGG GTCGTCCGTC TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG
```

FIG. 9K

```
                                                                         fnu4HI
                              nlaIII                                    sfiI mnlI
                             styI                                       haeIII/palI
                             ncoI                                  bsaJI bglI          ddeI
                       bslI  dsaI                                haeIII/palI bsaJI  mnlI  aluI                   mnlI
     bsrI  aciI        aciI  bsaJI                             mnlI  mnlI aciI    haeIII/palI                 mnlI
3201 CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG
     GGTCAAGGCG GGTAAGAGGC GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC

                                                          fnu4HI            hinPI
                      rmaI                              mcrI                hhaI/cfoI
                     styI                               eagI/xmaIII/eclXI  thaI
                     bsaJI                              eaeI               fnuDII/mvnI
                     blnI                           bsrBI          tru9I   bstUI             bspMI
                     avrII                       xhoI notI     tru9I       bsh1236I      scfI
                   haeIII/palI                   paeR7I haeIII/palI     hinPI     tru9I  pstI
                  stuI                           avaI fnu4HI   pacI     hhaI/cfoI mseI   bsgI
                  haeI                       maeIII taqI cfrI  mseI     bssHII   ahaIII/draI   maeIII
                 mnlI maeI              aluI     mnlI  aciI aciI   mseI ascI     swaI    sse8387I     aluI    bsrI
3301 AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AAAGCTGTTA CCTCGAGCGG CCGCTTAATT AAGGCGCGCC ATTTAAATCC TGCAGGTAAC AGCTTGGCAC
     TCCGAAAAAA CCTCCGGATC CGAAAACGTT TTTCGACAAT GGAGCTCGCC GGCGAATTAA TTCCGCGCGG TAAATTTAGG ACGTCCATTG TCGAACCGTG

                                           scrFI
                                           mvaI
                                           ecoRII
                                           dsaV
      haeIII/palI                          bstNI                                                    aluI
     eaeI                     bsrI         apyI[dcm+]        tru9I       fnu4HI                    pvuII            mboII
     cfrI             maeII maeIII        bsaJI   maeIII     mseI        bbvI  fokI                nspBII           earI/ksp632I
3401 TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCCTTC GCCAGCTGGC GTAATAGCGA
     ACCGGCAGCA AAATGTTGCA GCACTGACCC TTTTGGGACC GCAATGGGTT GAATTAGCGG AACGTCGTGT AGGGGGGAAG CGGTCGACCG CATTATCGCT

                                                                hinPI
                                                                hhaI/cfoI
                                                               nlaIV
                   sau3AI                                      narI
                   mboI/ndeII[dam-]                            kasI
                   dpnI[dam+]                                  hinlI/acyI
        sau96I     dpnII[dam-]                                 hgiCI
        haeIII/palI                                            haeII      aciI
        asuI      pvuI/bspCI                                   banI    sfaNI
      mnlI aciI   mcrI                         bglI            ahaII/bsaHI                    sfaNI   aciI
3501 AGAGGCCCGC ACCGATCGCC CTTCCCAACA GTTGCGTAGC CTGAATGGCG AATGGCGCCT GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA
     TCTCCGGGCG TGGCTAGCGG GAAGGGTTGT CAACGCATCG GACTTACCGC TTACCGCGGA CTACGCCATA AAAGAGGAAT GCGTAGACAC GCCATAAAGT
```

FIG. 9L

```
                                                                  aciI
                                    hinPI                      fnu4HI                  fnu4HI
                                    hhaI/cfoI                  aciI                  hinPI
                                   thaI                       thaI                   hhaI/cfoI
                                   fnuDII/mvnI                fnuDII/mvnI           thaI
                                   bstUI           hinPI      bstUI                 fnuDII/mvnI
                                   bsh1236I        hhaI/cfoI hinPI                  bstUI                   hinPI
                                rsaI      scfI fnu4HI   tru9I bsh1236I              bsh1236I     aciI            hhaI/cfoI
       aciI  maeII              csp6I   bslI   aciI     mseI hhaI/cfoI        maeIII  bbvI  maeIII            haeII
3601 CACCGCATAC GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
     GTGGCGTATG CAGTTTCGTT GGTATCATGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC

            hinPI                                                                              nlaIV
            hhaI/cfoI                                          mspI                            hgiJII
           haeII                                               hpaII                           bsp1286
         rmaI    bsrBI                                        naeI                             bmyI
         maeI    aciI            mboII                 maeII  cfr10I            aluI           banII        nlaIV
3701 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
     GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG

                      nlaIV                                       maeII    haeIII/palI
                      hgiCI   taqI                               draIII   sau96I                                 maeII
                      banI mnlI                   hphI       bsaAI    asuI                                  drdI
3801 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
     GCTAAATCAC GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAAACCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA GCGGGAAACT

           pleI          tru9I     pleI                              bslI
           hinfI  maeII  mseI      hinfI      bsrI             bslI   avaI
3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG CTATTCTTTT GATTTATAAG GGATTTTGCC
     GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCC GATAAGAAAA CTAAATATTC CCTAAAACGG

                                                          thaI                                       hgiAI/aspHI
                                                          fnuDII/mvnI                                bsp1286
                                                          bstUI                    maeII             bsiHKAI
                       tru9I                          tru9I                    tru9I                 bmyI   ddeI
                       mseI               tru9I       mseI    apoI tru9I       mseI                  apaLI/snoI  rsaI
            haeIII/palI           aluI    mseI    apoI    bsh1236I mseI    sspI  psp1406I            alw44I/snoI csp6I
4001 GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTATGGTG CACTCTCAGT
     CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCAAATGTT AAAATACCAC GTGAGAGTCA
```

FIG. 9M

```
                                                                                                              hinPI
                                                                                                              hhaI/cfoI
                                                                                                             thaI
                                                                             hinPI                           fnuDII/mvnI
                      aciI                               maeIII          fnu4HI                              bstUI
                     fnu4HI    tru9I                   maeII bsrI    nlaIII  hhaI/cfoI               nspBII  bsh1236I
                  sfaNI        mseI         aciI      bsaAI tth111I/aspI  bbvI            aciI       aciI  hgaI
4101 ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
     TGTTAGACGA GACTACGGCG TATCAATTCG GTTGAGGCGA TAGCGATGCA CTGACCCAGT ACCGACGCGG GGCTGTGGGC GGTTGTGGGC GACTGCGCGG

                                                                                                                  thaI
                                                                                                                  fnuDII/mvnI
                                                                                                                  bstUI
                                                                scrFI                                             bsh1236I
                         mspI                                   nciI                                             hinPI
                         hpaII                                  mspI                                             hhaI/cfoI
                        scrFI                            bsmAI           nspI                                   thaI
                        nciI     aciI                   esp3I  hpaII fnu4HI                                     fnuDII/mvnI
                        dsaV sfaNI                     bslI    dsaV aluI nspHI                                  bstUI
       drdI             cauII fokI             aluI maeIII      cauII bbvI nlaIII    mnlI   hphI      hphI      bsh1236I
4201 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
     GACTGCCCGA ACAGACGAGG GCCGTAGGCG AATGTCTGTT CGACACTGGC AGAGGCCCTC GACGTACACA GTCTCCAAAA GTGGCAGTAG TGGCTTTGCG

                                    mnlI
                                  haeIII/palI                                                    maeII
                      mboII      sau96I                                  nlaIII                 hinlI/acyI
                      bpuAI      asuI                             tru9I rcaI                    ahaII/bsaHI
       mnlI           bbsI      eco0109I/draII                    mseI  bspHI              ddeI aatII
4301 GCGAGGCAGT ATTCTTGAAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT
     CGCTCCGTCA TAAGAACTTC TGCTTTCCCG GAGCACTATG CGGATAAAAA TATCCAATTA CAGTACTATT ATTACCAAAG AATCTGCAGT CCACCGTGAA

                       nlaIV
                     aciI
                    thaI
                    fnuDII/mvnI
                    bstUI                                                    rcaI
                    bsh1236I                                                 bspHI
                   hinPI                                                 bsrBI    bsmAI
                   hhaI/cfoI                                             aciI nlaIII                        sspI
4401 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
     AAGCCCCTTT ACACGCGCCT TGGGGATAAA CAAATAAAAA GATTTATGTA AGTTTATACA TAGGCGAGTA CTCTGTTATT GGGACTATTT ACGAAGTTAT

                  mboII                                                 fnu4HI
                  earI/ksp632I                                          aciI                        hphI
4501 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG
     TATAACTTTT TCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGGAAT AAGGGAAAAA ACGCCGTAAA ACGGAAGGAC AAAAACGAGT GGGTCTTTGC
```

FIG. 9N

```
                                          hgiAI/aspHI
                                          bsp1286
                                 sau3AI   bsiHKAI                                 sau3AI  nspBII      sau3AI
                                 mboI/ndeII[dam-]                                 mboI/ndeII[dam-]    mboI/ndeII[dam-]
                                 dpnI[dam+] bmyI                                  dpnI[dam+]          dpnI[dam+]
                                 dpnII[dam-]                                     bstYI/xhoII          dpnII[dam-]
                             eco57I       apaLI/snoI                           bsrI dpnII[dam-]       alwI[dam-]
      hphI              sfaNI  mboII[dam-]  alw44I/snoI  maeIII taqI          alwI[dam-]     aciI  bstYI/xhoII         mboII
4601 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG
     GACCACTTTC ATTTTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT CGCCATTCTA GGAACTCTCA AAAGCGGGGC

                                                                                      scrFI
                                                                                      nciI
                                                      aciI                            mspI
                                                      thaI                            hpaII
                                                      fnuDII/mvnI                     dsaV
       maeII              hgiAI/aspHI                 bstUI                           cauII
      psp1406I            bsp1286   tru9I             bsh1236I                     hinlI/acyI
      xmnI                bsiHKAI   mseI              hinPI                        hgaI                            aciI
      asp700              bmyI    ahaIII/draI         hhaI/cfoI                    ahaII/bsaHI    bcgI   mcrI   fnu4HI
4701 AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTGATGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
     TTCTTGCAAA AGGTTACTAC TCGTGAAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACTACTGCG GCCCGTTCTC GTTGAGCCAG CGGCGTATGT

                                 rsaI
                                 csp6I   bsrI                                                    fnu4HI
        ddeI                    scaI hphI   maeIII      sfaNI     fokI   nlaIII                  bbvI        nlaIII
4801 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
     GATAAGAGTC TTACTGAACC AACTCATGAG TGGTCAGTGT CTTTTCGTAG AATGCCTACC GTACTGTCAT TCTCTTAATA CGTCACGACG GTATTGGTAC

                                                sau96I
                                                avaII                                              maeIII
                                          sau3AI  asuI                                           nlaIII
                  haeIII/palI             mboI/ndeII[dam-]                                      sau3AI
                  eaeI                    dpnI[dam+]                                            mboI/ndeII[dam-]   sau3AI
                  cfrI                    dpnII[dam-]                                           dpnI[dam+]         mboI/ndeII[dam-]
                 fnu4HI                  pvuI/bspCI                                             dpnII[dam-]        dpnI[dam+]
                 aciI                    mcrI    mnlI        aluI   aciI            nlaIII   alwI[dam-]        dpnII[dam-]
4901 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG
     TCACTATTGT GACGCCGGTT GAATGAAGAC TGTTGCTAGC CTCCTGGCTT CCTCGATTGG CGAAAAAACG TGTTGTACCC CCTAGTACAT TGAGCGGAAC
```

FIG. 9O

```
                                                                                                    hinPI
         mspI                                                                                       mstI
         hpaII                                                                                      aviII/fspI     bsrI
         bsaWI                                                                         fnu4HI  maeII hhaI/cfoI  tru9I
     nlaIV    aluI                                               maeIII      sfaNI     bbvI   psp1406I           mseI
5001 ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CAGCAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG
     TAGCAACCCT TGGCCTCGAC TTACTTCGGT ATGGTTTGCT GCTCGCACTG TGGTGCTACG GTCGTCGTTA CCGTTGTTGC AACGCGTTTG ATAATTGACC

                    mspI
                    hpaII                                                                      bglI
                    scrFI                                                                      sau96I
            aluI    nciI        tru9I      fokI                          sau96I                haeIII/palI
          rmaI      dsaV        mseI   bsrI        aciI                  avaII        hinPI    asuI    mspI
          maeI      cauII       aseI/asnI/vspI  mnlI                     asuI         hhaI/cfoI        hpaII
5101 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
     GCTTGATGAA TGAGATCGAA GGGCCGTTGT TAATTATCTG ACCTACCTCC GCCTATTTCA ACGTCCTGGT GAAGACGCGA GCCGGGAAGG CCGACCGACC

                    mspI             thaI
                    hpaII            fnuDII/mvnI              haeIII/palI
                    cfr10I           bstUI                   sau96I
                 nlaIV  hphI      bsmAI aciI       fnu4HI  nlaIV
               gsuI/bpmI         bsaI bsh1236I     bbvI bsrI asuI         mnlI
5201 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA
     AAATAACGAC TATTTAGACC TCGGCCACTC GCACCCAGAG CGCCATAGTA ACGTCGTGAC CCCGGTCTAC CATTCGGGAG GGCATAGCAT CAATAGATGT

                                               ddeI
                                            sau3AI        nlaIV
       pleI                                 mboI/ndeII[dam-]  mnlI
       hinfI                                dpnI[dam+]    hgiCI       tru9I
     eam1105I           fokI                dpnII[dam-]   banI        mseI        maeIII
5301 CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
     GCTGCCCCTC AGTCCGTTGA TACCTACTTG CTTTATCTGT CTAGCGACTC TATCCACGGA GTGACTAATT CGTAACCATT GACAGTCTGG TTCAAATGAG

                                                   rmaI      sau3AI
                                               sau3AI hphI   mboI/ndeII[dam-]
                                               mboI/ndeII[dam-]
                                               dpnI[dam+]    dpnI[dam+]
                                        tru9I  dpnII[dam-]   dpnII[dam-]
                 tru9I                ahaIII/draI maeI       alwI[dam-]          nlaIII            maeII
                 mseI          tru9I        bstYI/xhoII  bstYI/xhoII             rcaI            tru9I
               ahaIII/draI    mseI mseI     alwI[dam-] mboII[dam-]               bspHI           mseI
5401 ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
     TATATATGAA ATCTAACTAA ATTTTGAAGT AAAAATTAAA TTTTCCTAGA TCCACTTCTA GGAAAAACTA TTAGAGTACT GGTTTTAGGG AATTGCACTC
```

FIG. 9P

```
                                                sau3AI
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]  sau3AI           thaI
                                                dpnII[dam-] mboI/ndeII[dam-]
                                               bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                         sau3AI alwI[dam-]  dpnII[dam-]      bstUI
                                         mboI/ndeII[dam-]   alwI[dam-]       bsh1236I
                     hgaI                dpnI[dam+] mboII[dam-]             hinPI       fnu4HI
                 ddeI                    dpnII[dam-]       bstYI/xhoII      hhaI/cfoI   bbvI
5501 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC
     AAAAGCAAGG TGACTCGCAG TCTGGGGCAT CTTTTCTAGT TTCCTAGAAG AACTCTAGGA AAAAAAGACG CGCATTAGAC GACGAACGTT TGTTTTTTTG

                                   sau3AI
                                   mboI/ndeII[dam-]
                                   dpnI[dam+]
                                   dpnII[dam-]
                                  alwI[dam-]
                  aciI            mspI                                 bsrI           hinPI
       aciI   nspBII              hpaII      aluI                   maeIII    eco57I  hhaI/cfoI
5601 CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
     GTGGCGATGG TCGCCACCAA ACAAACGGCC TAGTTCTCGA TGGTTGAGAA AAAGGCTTCC ATTGACCGAA GTCGTCTCGC GTCTATGGTT TATGACAGGA

                                                                                             fnu4HI
                                                                                        alwNI    bbvI
      rmaI             haeIII/palI                                                    bsrI  fnu4HI
      maeI      bslI   haeI                 scfI      aciI        mnlI            maeIII    bbvI     bsrI
5701 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
     AGATCACATC GGCATCAATC CGGTGGTGAA GTTCTTGAGA CATCGTGGCG GATGTATGGA GCGAGACGAT TAGGACAATG GTCACCGACG ACGGTCACCG

                       scrFI                                       aciI                    hgiAI/aspHI
                       nciI                                      nspBII                    bsp1286
                       mspI                       mspI          fnu4HI                     bsiHKAI
                       hpaII                      hpaII         bbvI                       bmyI
                       dsaV     pleI             bsaWI        hinPI mcrI                   apaLI/snoI
                       cauII    hinfI        maeIII           hhaI/cfoI                    alw44I/snoI  aluI
5801 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
     CTATTCAGCA CAGAATGGCC CAACCTGAGT TCTGCTATCA ATGGCCTATT CCGCGTCGCC AGCCCGACTT GCCCCCCAAG CACGTGTGTC GGGTCGAACC
```

FIG. 9Q

```
                                                                                                       mspI
                                                             hinPI                                     hpaII
                                                             hhaI/cfoI                                 bslI    fnu4HI
                           ddeI     scfI                    haeII                         aciI        bsaWI    aciI
5901 AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
     TCGCTTGCTG GATGTGGCTT GACTCTATGG ATGTCGCACT CGTAACTCTT TCGCGGTGCG AAGGGCTTCC CTCTTTCCGC CTGTCCATAG GCCATTCGCC

                                          scrFI
                                          mvaI          scrFI
                                          ecoRII  mvaI
                                          dsaV    ecoRII
                                          bstNI         dsaV
                        hinPI  mnlI       bsaJI         bstNI                                                  taqI
                        hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]                             mnlI drdI    hgaI
6001 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
     GTCCCAGCCT TGTCCTCTCG CGTGCTCCCT CGAAGGTCCC CCTTTGCGGA CCATAGAAAT ATCAGGACAG CCCAAAGCGG TGGAGACTGA ACTCGCAGCT

                                                                                   haeIII/palI
                                                               haeIII/palI     scrFI
                                                             fnu4HI            mvaI
                                                             aciI              ecoRII
                                                             thaI bslI         dsaV                    nlaIII
                                                             fnuDII/mvnI       bstNI bslI                   nspI
                                nlaIV                        bstUI             apyI[dcm+]       haeIII/palI nspHI
            sfaNI             aciI                           bsh1236I     nlaIV haeI        haeI        aflIII
6101 TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
     AAAAACACTA CGAGCAGTCC CCCCGCCTCG GATACCTTTT TGCGGTCGTT GCGCCGGAAA AATGCCAAGG ACCGGAAAAC GACCGGAAAA CGAGTGTACA

                                                                                    fnu4HI              fnu4HI
                                                                                    bbvI                bbvI pleI
                           tfiI                                              bsrBI   aciI            hinPI   hinfI
                           hinfI                   aciI          aluI        aciI  fnu4HI       mcrI hhaI/cfoI
6201 TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
     AGAAAGGACG CAATAGGGGA CTAAGACACC TATTGGCATA ATGGCGGAAA CTCACTCGAC TATGGCGAGC GGCGTCGGCT TGCTGGCTCG CGTCGCTCAG
```

FIG. 9R

```
                                                                    thaI
                                                                    fnuDII/mvnI
                                                                    bstUI
                                                                    bsh1236I
                                                                  hinPI
                                                                  hhaI/cfoI
                                                                 thaI
                                                                 fnuDII/mvnI
                      sapI  hinPI                                bstUI               tru9I    aluI
                      mboII hhaI/cfoI                            bsh1236I haeIII/palI         pvuII
                      earI/ksp632I                   mnlI      bslI      eaeI  tfiI aseI/asnI/vspI
            mnlI    aciI    haeII                 aciI         aciI      cfrI  hinfI mseI   nspBII                  bsrI
6301 AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG
     TCACTCGCTC CTTCGCCTTC TCGCGGGTTA TGCGTTTGGC GGAGAGGGGC GCGCAACCGG CTAAGTAATT AGGTCGACCG TGCTGTCCAA AGGGCTGACC

                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                                                                          dsaV
                                  tru9I                            nlaIV bstNI
                    hinPI         mseI      maeIII                 hgiCI apyI[dcm+]              mspI
        aciI        hhaI/cfoI    aseI/asnI/vspI mnlI               banI bsaJI                    hpaII
6401 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
     TTTCGCCCGT CACTCGCGTT GCGTTAATTA CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT

                                                                       tru9I
                                                                       mseI
                                                                      aseI/asnI/vspI
          aciI                                                     xmnI
        bsrBI                          aluI       nlaIII           asp700
6501 TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTAA
     AACACTCGCC TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAATT

>length: 6557
```

FIG. 10A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286                                                                                      thaI
       bsiHKAI                                                                                      fnuDII/mvnJ
       bmyI                       rmaI    tru9I                                                     bstUI
       banII                      maeI    mseI                                                      bsh1236I
     taqI                        speI    aseI/asnI/vspI                          bslI              aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                                maeII
                sau96I                                   hinlI/acyI
                haeIII/palI     aciI                     ahaII/bsaHI
                asuI  apyI[dcm+]               aciI     aatII          maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                      maeII
      hinlI/acyI                                                                                 hinlI/acyI
      ahaII/bsaHI                                    rsaI                       rsaI           ahaII/bsaHI
      aatII                              bglI         csp6I            ndeI       csp6I          aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                     nlaIII
         bglI  dsaV                                                                           styI
        sau96I bstNI            rsaI                                        maeII                  ncoI
        haeIII/palI             csp6I                               rsaI   snaBI                   dsaI hphI   aciI
        asuI   apyI[dcm+]   bsrI  nlaIII                            csp6I  bsaAI                   bsaJI    sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 10B

```
                                                                                   maeII
                                                                                  hinlI/acyI               nlaIV
               rsaI                          pleI                                 ahaII/bsaHI              hgiCI
               csp6I                  aciI   hinfI                bsmAI           aatII                    banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                          aluI
                                                                                                         sstI
                                                                                                         sacI
                                                                                                         hgiJII
                                                                                                         hgiAI/aspHI
                                                                                                         ecl136II
                                                                                                         bsp1286
                                                                                                         bsiHKAI
                                                                             rsaI                        bmyI
                                maeIII    aciI       hgaI        aciI        csp6I     mnlI              banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                haeIII/palI
                                                                                               mcrI
                                                                                               eagI/xmaIII/eclXI
                                                                                               eaeI
                                                                                               cfrI
                                                                                              fnu4HI
                                                                                              aciI
                                                                                             thaI
                              esp3I                                                          fnuDII/mvnI
                         scrFI                                            sau96I           sacII/sstII
                         mvaI bsmAI                                             avaII            nspBII
                         ecoRII                                           asuI             kspI  scrFI
                         dsaV                                            nlaIV             dsaI  nciI
                         bstNI  hinlI/acyI                                   scrFI          bglI bslI  mspI
                         apyI[dcm+]                                          nciI     sau3AI mnlI bstUI
                    sau3AI gsuI/bpmI                                         mspI     mboI/ndeII[dam-] hpaII
                    mboI/ndeII[dam-]                                 mboII   hpaII    dpnI[dam+] bsaJI dsaV
                    dpnI[dam+]  hgaI  fokI                           bpuAI   dsaV     dpnII[dam-] bsh1236I
                    dpnII[dam-] ahaII/bsaHI                 mnlI     bbsI    cauII    alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 10C

```
         tfiI                                                                                    fnu4HI
       aciI                                                                                      aciI
      thaI hinfI                                                  bstXI                         thaI
      fnuDII/mvnI                       aciI                    sau96I    styI                 fnuDII/mvnI tru9I
      bstUI                       maeII rsaI          pleI scfI haeIII/palI                    bstUI       mseI
      bsh1236I                maeIII    csp6I     scfI hinfI    asuI      bsaJI               bsh1236I   aseI/asnI/vspI
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT

                                                                                                   sau96I
                                                                                                   avaII
                                                                                                    asuI
                                                                                                 scrFI
                                                                                                 mvaI
                                                                                                 ecoRII
                                                                                                 dsaV
                                                                                                 bstNI
                                  maeIII                                                         apyI[dcm+]
                                 hphI   scfI        fokI                                     bslI bsaJI
 801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
     GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG

                                                  hinlI/acyI
                                                  ahaII/bsaHI
                                                  aatII
                                                thaI                  taqI[dam-]
                                                fnuDII/mvnI          sau3AI                  bsmAI
            aluI  taqI                          bstUI                mboI/ndeII[dam-]       esp3I
   mnlI    hindIII                             aciI maeII           dpnI[dam+]          tth111I/aspI
   bsaJI   ddeI    claI/bsp106              hphI bsh1236I    taqI    dpnII[dam-] taqI   hgaI         sfaNI
 901 ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT
     TGGAGCCAAG ATTCGAATAG CTATACTTTT TCGGACTTGA GTGGCGCTGC AGACAGCTCT TCAAAGACTA GCTTTTCAAG CTGTCGCAGA GGCTGGACTA

                                                                                            hinPI
     aluI              tfiI                                                              fnu4HI
    fnu4HI             hinfI                 taqI                                        bbvI
    bbvI      mnlI  mboII               aluI           mnlI               aciI         aluI hhaI/cfoI
1001 GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA
     CGTCGAGAGC CTCCCGCTTC TTAGAGCACG AAAGTCGAAG CTACATCCTC CCGCACCTAT ACAGGACGCC CATTTATCGA CGCGGCTACC AAAGATGTTT
```

FIG. 10D

```
                                hinPI
                                hhaI/cfoI    mspI
                               thaI          hpaII
                              aciI          mroI
                            haeIII/palI     bspMII
                           mcrI fnuDII/mvnI bspEI
     sau3AI                eagI/xmaIII/eclXI bsaWI
     mboI/ndeII[dam-]      eaeI bstUI     tfiI
     dpnI[dam+]            cfrI bsh1236I  hinfI               ecoRI
     dpnII[dam-]       sfaNI  fnu4HI  bslI  accIII               apoI                            sfaNI  aciI
1101 GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC
     CTAGCAATAC AAATAGCCGT GAAACGTAGC CGGCGCGAGG GCTAAGGCCT TCACGAACTG TAACCCCTTA AGTCGCTCTC GGACTGGATA ACGTAGAGGG

                                                                                                 mcrI
                                                                                                 eagI/xmaIII/eclXI
                                                                                                 eaeI
                                                                              styI              fnu4HI
       hgiAI/aspHI                                                      thaI   ncoI        sau3AI  aciI
       bsp1286                                                          fnuDII/mvnI        mboI/ndeII[dam-]
       bsiHKAI                                                    fnu4HI  bstUI   dsaI     dpnI[dam+]      sau3AI
       bmyI                                                       bbvI  mcrI     haeIII/palI dpnII[dam-]   mboI/ndeII[dam-]
       apaLI/snoI                                               scfI  mspI bsh1236I bsaJI sfaNI  fnu4HI   dpnI[dam+]
       alw44I/snoI    maeII                            nspBII   pstI  hpaII    mnlI nlaIII  pvuI/bspCI haeIII/palI
     bslI draIII    maeIII        bspMI                aciI     bsgI  cfr10I aciI haeI  fokI  mcrI bbvI cfrI dpnII[dam-]
1201 GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA
     CGGCACGTGT CCCACAGTGC AACGTTGTGG ACGGACTTTG GCTTGACGGG CGACAAGACG TCGGCCAGCG CCTCCGGTAC CTACGCTAGC GACGCCGGCT

                                  sau96I                                                    thaI       sau3AI
                                  avaII                                                     fnuDII/mvnI      nlaIII
                                  asuI                                                      bstUI      mboI/ndeII[dam-]
                           sau96I  rsrII/cspI                                               bsh1236I   dpnI[dam+]
                  aciI     haeIII/palI  aciI    tfiI                                       hinPI       dpnII[dam-]
      ddeI       bsrBI     asuI       cpoI      hinfI              nlaIII           ndeI  hhaI/cfoI    alwI[dam-]
1301 TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG
     AGAATCGGTC TGCTCGCCCA AGCCGGGTAA GCCTGGCGTT CCTTAGCCAG TTATGTGATG TACCGCACTA AAGTATACGC GCTAACGACT AGGGGTACAC

                                            hinPI                                                       draIII
                                            hhaI/cfoI                                                   nlaIV
                                           thaI                                      bsaJI              hgiCI
                                           fnuDII/mvnI                            haeIII/palI           banI
                             tth111I/aspI  bstUI                        sfaNI     sau96I               mspI
        bsrI              drdI         hgaI  bsh1236I    taqI      aluI          asuI mnlI      bslI   hpaII
1401 TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC
     ATAGTGACCG TTTGACACTA CCTGCTGTGG CAGTCACGCA GGCAGCGCGT CCGAGAGCTA CTCGACTACG AAACCCGGCT CCTGACGGGG CTTCAGGCCG
```

FIG. 10E

```
              aciI
             thaI
             fnuDII/mvnI
       hgiAI/aspHI
       bsp1286
       bsiHKAI                                          aciI
       bmyI  bstUI                                     fnu4HI
       apaLI/snoI                                     haeIII/palI
       alw44I/snoI                                   eaeI        aciI      gsuI/bpmI           tfiI bslI
   mnlI        bsh1236I   nlaIV                      cfrI       nspBII    bsrI     mnlI        hinfI
1501 ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA
     TGGAGCACGT GCGCCTAAAG CCGAGGTTGT TACAGGACTG CCTGTTACCG GCGTATTGTC GCCAGTAACT GACCTCGCTC CGCTACAAGC CCCTAAGGGT

                                                                                                      fnu4HI
                                                                                                     thaI
                                                                                                     fnuDII/mvnI
                                                                                                     bstUI
                                                                                                     bsh1236I
                                                                                                    sacII/sstII
                                                                                                    nspBII
                                                                                                    kspI
                                                                                   mspI             dsaI
                                                                                   hpaII            bsaJI
                                                                                  mroI              aciI
                                                                                  bspMII           fnu4HI
                                                                                  bspEI           sau3AI aciI
                                     dsaI                                         bsaWI           mboI/ndeII[dam-]
                                   haeIII/palI                  rsaI     aciI    fokI             dpnI[dam+]
                            mboII  mnlI bsaJI          fnu4HI    csp6I  bsrBI   sfaNI    aluI     dpnII[dam-]
     mnlI         mboII   gsuI/bpmI                    bbvI    maeII   taqI   mnlI  accIII       alwI[dam-]
1601 ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG
     TATGCTCCAG CGGTTGTAGA AGAAGACCTC CGGCACCAAC CGAACATACC TCGTCGTCTG CATGAAGCTC GCCTCCGTAG GCCTCGAACG TCCTAGCGGC

       scrFI
       nciI
       mspI
       hpaII                                                                     aluI
       dsaV                                                                     fnu4HI                   hgaI
       cauII                                                                    bbvI      hinPI    taqI  drdI
   nlaIV           aciI                             aluI    hincII/hindII taqI sfaNI      hhaI/cfoI  sfaNI
1701 CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG
     GCCGAGGCCC GCATATACGA GGCGTAACCA GAACTGGTTG AGATAGTCTC GAACCAACTG CCGTTAAAGC TACTACGTCG AACCCGCGTC CCAGCTACGC
```

FIG. 10F

```
           nlaIV                                                          haeIII/palI
          mspI                                                           mcrI
          hpaII  scrFI                                                   eagI/xmaIII/eclXI
          bslI   nciI                                                    eaeI
         mroI    mspI                                                    cfrI
         bspMII  hpaII                                                 fnu4HI
         bspEI[dam-]                                                   aciI
         bsaWI   dsaV                                                 thaI
         accIII[dam-]                                                 fnuDII/mvnI
        sau3AI   cauII                                                bstUI
        mboI/ndeII[dam-]                                              bsh1236I    sau96I              rsaI
        dpnI[dam+]                                                    hinPI       avaII               csp6I
        dpnII[dam-]               rsaI                               hhaI/cfoI   asuI               scaI
        alwI[dam-]                csp6I           aciI
1801 ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA
     TGCGTTAGCA GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC CTGGCTACCG ACACATCTTC ATGAGCGGCT

                                                                             scrFI
                                                                             nciI
                                                                             mspI
                                                                             hpaII
                                                                             dsaV
                                                                            xmaI/pspAI
                                                                            smaI
                                                                            scrFI
                                                                            nciI
                                                                            dsaV
                                                                            cauII
                                                                            bsaJI
                                                                            avaI
                                                                           bsaJI
                                                                         sau3AI                              sau96I
                                                                         mboI/ndeII[dam-]           aciI     haeIII/palI
                                                                         dpnI[dam+]                fnu4HI    asuI
                                                                         dpnII[dam-]               bglI  nlaIII
                                                                         alwI[dam-]               sfiI  styI
                                                                        nlaIV cauII              eaeI   ncoI
        hinlI/acyI                                           mcrI       bstYI/xhoII              cfrI   dsaI
        hgaI                  mnlI                          bslI        bamHI bsaJI ecoRI     taqI haeIII/palI
        ahaII/bsaHI         bsaJI                        sfaNI          alwI[dam-]  apoI     claI/bsp106 bsaJI
1901 TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC
     ATCACCTTTG GCTGCGGGGT CGTGAGCAGG CTCCCGTTTC CTTATCTCAT CTACGGCTGG CTTCCTAGGG GCCCCTTAAG TTAGCTACCG GCGGTACCGG
```

FIG. 10G

```
              aluI
             fnu4HI
             bbvI          maeIII                sfaNI   apoI                                      rmaI
                                                                                             bsmI maeI
2001 CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG
     GTTGAACAAA TAACGTCGAA TATTACCAAT GTTTATTTCG TTATCGTAGT GTTTAAAGTG TTTATTTCGT AAAAAAAGTG ACGTAAGATC AACACCAAAC

                                    sau3AI
                                    mboI/ndeII[dam-]
                                    dpnI[dam+]
                                    dpnII[dam-]
                                   pvuI/bspCI
                                   mcrI
                                  taqI[dam-]  tru9I                   haeIII/palI
                                 claI/bsp106[dam-]                   haeI
                                sau3AI       mseI       fnu4HI styI
                                mboI/ndeII[dam-]        bbvI   ncoI
                                dpnI[dam+] xmnI        hinPI   dsaI
                                dpnII[dam-]  aseI/asnI/vspI    bsaJI
                           nlaIII  alwI[dam-]  asp700  hhaI/cfoI nlaIII         mnlI      mnlI
2101 TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA
     AGGTTTGAGT AGTTACATAG AATAGTACAG ACCTAGCTAG CCCTTAATTA AGCCGCGTCG TGGTACCGGA CTTTATTGGA GACTTTCTCC TTGAACCAAT

      rsaI                                                              nlaIV
      csp6I                                                           scrFI                             sfaNI
     nlaIV                                                            mvaI                                  ppu10I
     kpnI                                                             ecoRII                              nsiI/avaIII
     hgiCI                                                            dsaV                               nlaIII
     banI                        aluI                                 bstNI                                 sphI
     asp718    mnlI             pvuII                                 apyI[dcm+]                            nspI
     acc65I  ddeI  aciI         nspBII                               bsaJI                                  nspHI
2201 GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     CCATGGAAGA CTCCGCCTTT CTTGGTCGAC ACCTTACACA CAGTCAATCC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG

                                             nlaIV
                scrFI        scrFI
                mvaI               mvaI                                       ppu10I
                ecoRII       ecoRII                                 nsiI/avaIII
                dsaV         dsaV                                  nlaIII
                bstNI              bstNI                                     sphI
                apyI[dcm+]         apyI[dcm+]                                nspI sfaNI
               sexAI              bsaJI                                      nspHI                         aciI
2301 ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC
     TAGAGTTAAT CAGTCGTTGG TCCACACCTT TCAGGGGTCC GAGGGGTCGT CCGTCTTCAT ACGTTTCGTA CGTAGAGTTA ATCAGTCGTT GGTATCAGGG
```

FIG. 10H

```
                                                                                                               fnu4HI
                                                                                                               bglI
                                                                    nlaIII                                    sfiI
                                                                   styI                                       haeIII/palI
                                                                   ncoI                                     mnlI
                         aciI              bsrI               bslI dsaI                                 haeIII/palI
                aciI fokI              aciI       aciI        aciI bsaJI                             mnlI bsaJI aciI
2401 GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC
     CGGGGATTGA GGCGGGTAGG GCGGGGATTG AGGCGGGTCA AGGCGGGTAA GAGGCGGGGT ACCGACTGAT TAAAAAAAAT AAATACGTCT CCGGCTCCGG

                                                                                               scrFI
                                                                                               nciI
                                                                                               mspI
                                                                                               hpaII
                                                                                               dsaV
                                                            styI                             haeIII/palI
                                                            bsaJI                          mcrI
                                                            blnI                           eagI/xmaIII/eclXI
                                                         haeIII/palI            rmaI       eaeI
          haeIII/palI                                   stuI rmaI               maeI       cfrI
      mnlI      ddeI                      mnlI          haeI maeI              nheI       mspI cauII
      bsaJI mnlI   aluI                mnlI            mnlI avrII             aluI aluI   hpaII
2501 GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG
     CGGAGCCGGA GACTCGATAA GGTCTTCATC ACTCCTCCGA AAAAACCTCC GGATCCGAAA ACGTTTTTCG ATCGAATAGG CCGGCCCTTG CCACGTAACC

           tfiI
           hinfI                                                                             fnu4HI
        aciI                                                                                 aciI
       thaI                                                       bstXI                    thaI
       fnuDII/mvnI                         aciI          scfI  sau96I    styI              fnuDII/mvnI  tru9I
       bstUI                pleI        rsaI         pleI      haeIII/palI                 bstUI        mseI
       bsh1236I             hinfI       csp6I  scfI  hinfI     asuI      bsaJI             bsh1236I   aseI/asnI/vspI
2601 AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA
     TTGCGCCTAA GGGGCACGGT TCTCAGTCCA TTCATGGCGG ATATCTCAGA TATCCGGGTG GGGGAACCGA AGCAATCTTG CGCCGATGTT AATTATGTAT
```

FIG. 10I

sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
taqI[dam-]
claI/bsp106[dam-]
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
alwI[dam-]
fokI
nlaIII
styI
pflMI
ncoI
sfaNI ecoRI dsaI
fnu4HI taqI apoI bslI fokI
bbvI claI/bsp106 bsaJI nlaIII fokI rmaI maeI
hphI maeIII bspMI
bstEII hphI bsrI
mnlI
aciI
hgiJII
hgiAI/aspHI
ec11361I
bsp1286
bsiHKAI
bmyI
banII
avaI
sau96I
avaII
asuI
scrFI
mvaI
ecoRII
dsaV
bstNI
apyI[dcm+]
bslI bsaJI
rsaI
gsuI/bpmI
bsrI csp6I
hgaI
taqI
thaI maeI
fnuDII/mvnI
bstUI nheI
mnlI bsh1236I aluI
bsaJI nruI aluI
aluI
pvuII tth111I/aspI
ecoRV nspBII bsrI
hphI aluI nlaIII
bsrI
2701 ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC CACAGGTGTC CACTCCCAGG TCCAACTGCA CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
TGGAAAACCT AGCTAGGATG ACTGTGACTG TAGGTGAAAA AGAAAAAGAG GTGTCCACAG GTGAGGGTCC AGGTTGACGT GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT
2801 TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
AACCCGACGT AGCTAACTTA AGGTGGTACC CTACCAGTAC ATAGTAGGAA AAAGATCATC GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT
2901 GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG
CAGGGGCTCG AGGGACAGGC GGAGACACCC GCTATCCCAG TGGTAGTGGA CGGCACGGTC AGTCTCGCAG CTAATGCTAC CACTATCGAT GTACTTGACC

FIG. 10J

```
                                                                                                                         mspI
                                                                                                                         hpaII
                                                                                                                         bslI
                                                                                     gsuI/bpmI                          bsaWI
                                                                                     scrFI                     sau3AI
                                                                                     mvaI                           mboI/ndeII[dam-]
                                                                          haeIII/palI                               dpnI[dam+]
           scrFI                                                 fnu4HI         ecoRII                    dpnII[dam-]
           mvaI                                                        aciI          dsaV                      alwI[dam-]
           ecoRII                                               thaI mnlI       bstNI                         nlaIV
           dsaV                                                 fnuDII/mvnI     apyI[dcm+]   pleI             bstYI/xhoII
           bstNI         aluI                                         bstUI        rsaI     pleI gsuI/bpmI             bamHI
           apyI[dcm+]                                                 bsh1236I     csp6I    hinfI      hinfI             alwI[dam-]
3001 TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA
     ATAGTTGTCT TTGGTCCTTT TCGAGGCTTT GATGACTAAA TGCGCCGGAG CATGGACCTC AGACCTCAGG GAAGAGCGAA GAGACCTAGG CCAAGACCCT

                                                                                                 sau3AI                       styI
                                                                                                 mboI/ndeII[dam-]              bsaJI
                                                                                                 dpnI[dam+]                  rsaI
                                                                                                 dpnII[dam-]                 csp6I
                                                                                                 alwI[dam-]                 nlaIV
                                            fnu4HI  mboII                                       nlaIV                       kpnI
                                            bbvI    bpuAI                                       bstYI/xhoII                 hgiCI
                                          scfI      bbsI                                        bamHI  rsaI                 banI
                                          pstI   mspI                                         mnlI    csp6I                 asp718
                                          bsgI   hpaII                                 maeIII  alwI[dam-]                   acc65I
3101 CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC
     GCCTAAAGTG AGACTGGTAG TCGTCAGACG TCGGCCTTCT GAAGCGTTGA ATAATGACAG TCGTTTCAGT GCTCCTAGGC ATGTGTAAAC CTGTCCCATG

        sau3AI
        mboI/ndeII[dam-]                    mboII
        dpnI[dam+]          fnu4HI         bpuAI         aciI
        dpnII[dam-]         bbvI           bbsI    mboII                                         mnlI
3201 CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
     GTTCCACCTC TAGTTTGCTT GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT GACGGAGACA ACACACGGAC

                                                                                           scrFI
                                                                                           mvaI
                                                                                           ecoRII
                                                                                           dsaV
                                                                                           bstNI
                                   haeIII/palI                                             apyI[dcm+]
      xmnI                        haeI      rsaI                    mnlI                                   
      asp700                     mnlI       csp6I                   bslI        maeIII bsaJI        maeIII
3301 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA
     GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT
```

FIG. 10K

```
                                                                                                              sstI
                                                                                                              sacI
                                                                                                              hgiJII
                                                                                                              hgiAI/aspHI
                                                                                                              ecl136II
                                                                                                              bsp1286
                                                                                                              bsiHKAI
                                                                                                              bmyI
                                                                                                          haeIII/palI
                                                                                                         sau96I aluI
                                                                                                         asuI  banII
                                     ddeI                                                               eco0109I/draII
                                    celII/espI
                ddeI fnu4HI         bpu1102I                                          hphI
          scfI  mnlI  bbvI       hgaI                                accI             maeIII    alwNI   ddeI
3401 AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA GTCACCCATC AGGGCCTGAG
     TCCTGTCGTG GATGTCGGAG TCGTCGTGGG ACTGCGACTC GTTTCGTCTG ATGCTCTTTG TGTTTCAGAT GCGGACGCTT CAGTGGGTAG TCCCGGACTC

                                                                  sau96I
                                                               nlaIII
                                                           aciI   haeIII/palI
                                                         fnu4HI   asuI
                                                         bglI styI
                                                 aluI   sfiI  ncoI
                                                hindIII eaeI  dsaI                       aluI
                                              tru9I     cfrI  bsaJI                     fnu4HI
       maeIII    aluI                         mseI   taqI haeIII/palI                   bbvI          maeIII
3501 CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAA GCTTCGATGG CCGCCATGGC CCAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG
     GAGCGGGCAG TGTTTCTCGA AGTTGTCCCC TCTCACAATT CGAAGCTACC GGCGGTACCG GGTTGAACAA ATAACGTCGA ATATTACCAA TGTTTATTTC

                                                                                                              sau3AI
                                                                                                              mboI/ndeII[dam-]
                                                                                                              dpnI[dam+]
                                                                                                              dpnII[dam-]
                                                                                                             pvuI/bspCI
                                                                                                             mcrI
                                                                                                            taqI[dam-]
                                                                                                           claI/bsp106[dam-]
                                                                                                          sau3AI
                                                                                                          mboI/ndeII[dam-]
                                                                                                          dpnI[dam+]
                                                          rmaI                                            dpnII[dam-]
      sfaNI    apoI                                 bsmI maeI                                   nlaIII    alwI[dam-]
3601 CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCGAT
     GTTATCGTAG TGTTTAAAGT GTTTATTTCG TAAAAAAAGT GACGTAAGAT CAACACCAAA CAGGTTTGAG TAGTTACATA GAATAGTACA GACCTAGCTA
```

FIG. 10L

```
                                                                         rsaI
                                 haeIII/palI                             csp6I
                                haeI                                    nlaIV
           tru9I      fnu4HI styI                                       kpnI
           mseI       bbvI   ncoI                                       hgiCI
          aseI/asnI/vspI     dsaI                                       banI                       aluI
        xmnI        hinPI    bsaJI                                      asp718     mnlI           pvuII
        asp700      hhaI/cfoI nlaIII         mnlI      mnlI             acc65I  ddeI  aciI        nspBII
3701 CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA AGAACCAGCT GTGGAATGTG
     GCCCTTAATT AAGCCGCGTC GTGGTACCGG ACTTTATTGG AGACTTTCTC CTTGAACCAA TCCATGGAAG ACTCCGCCTT TCTTGGTCGA CACCTTACAC

                                  nlaIV                                                                        nlaIV
                               scrFI                           sfaNI               scrFI         scrFI
                               mvaI                                ppu10I                mvaI                mvaI
                               ecoRII                        nsiI/avaIII           ecoRII        ecoRII
                               dsaV                         nlaIII                 dsaV          dsaV
                               bstNI                             sphI                    bstNI               bstNI
                               apyI[dcm+]                        nspI                    apyI[dcm+]          apyI[dcm+]
                              bsaJI                              nspHI                  sexAI               bsaJI
3801 TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG
     ACAGTCAATC CCACACCTTT CAGGGGTCCG AGGGGTCGTC CGTCTTCATA CGTTTCGTAC GTAGAGTTAA TCAGTCGTTG GTCCACACCT TTCAGGGGTC

                                    ppu10I
                                    nsiI/avaIII
                                   nlaIII
                                  sphI
                                  nspI sfaNI                                                                  bsrI
                                  nspHI                              aciI          aciI fokI aciI          aciI
3901 GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA CTCCGCCCAG
     CGAGGGGTCG TCCGTCTTCA TACGTTTCGT ACGTAGAGTT AATCAGTCGT TGGTATCAGG GCGGGGATTG AGGCGGGTAG GGCGGGGATT GAGGCGGGTC

                                                                    fnu4HI
                                                                    bglI
                         nlaIII                                    sfiI
                        styI                                       haeIII/palI
                        ncoI                                     mnlI    mnlI     ddeI
                   bslI dsaI                                 haeIII/palI bsaJI mnlI  aluI                     mnlI
       aciI        aciI bsaJI                             mnlI bsaJI aciI   haeIII/palI                    mnlI
4001 TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC
     AAGGCGGGTA AGAGGCGGGG TACCGACTGA TTAAAAAAAA TAAATACGTC TCCGGCTCCG GCGGAGCCGG AGACTCGATA AGGTCTTCAT CACTCCTCCG
```

FIG. 10M

```
              styI                                                                                      scrFI
              bsaJI                                                                                     mvaI
              blnI                                                                                      ecoRII
           haeIII/palI              tru9I            haeIII/palI                                        dsaV
          stuI rmaI                 mseI            eaeI                                                bstNI
          haeI maeI                hpaI  aluI       cfrI                     maeIII                     apyI[dcm+]      tru9I
         mnlI avrII           aluI hincII/hindII  bsrI                  maeII    bsrI                   bsaJI  maeIII   mseI
4101 TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTGTTAACAG CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT
     AAAAAACCTC CGGATCCGAA AACGTTTTTC GACAATTGTC GAACCGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA

                                                                       sau3AI
                                                                       mboI/ndeII[dam-]
                                                             sau96I    dpnI[dam+]
                                                             haeIII/palI
                                     aluI                 mnlI  aciI   dpnII[dam-]
           fnu4HI                    pvuII              mboII asuI    pvuI/bspCI
           bbvI  fokI                nspBII             earI/ksp632I  mcrI                             bglI
4201 TAATCGCCTT GCAGCACATC CCCCCTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGTAGCCT GAATGGCGAA
     ATTAGCGGAA CGTCGTGTAG GGGGGAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCATCGGA CTTACCGCTT

  hinPI
  hhaI/cfoI
 nlaIV                                                                                  hinPI
 narI                                                                                   hhaI/cfoI
 kasI                                                                                  thaI
 hinlI/acyI                                                                            fnuDII/mvnI             hinPI
 hgiCI                                                                                 bstUI         hinPI     hhaI/cfoI
 haeII      aciI                                                                       bsh1236I      hhaI/cfoI
 banI   sfaNI                                                                       rsaI      scfI fnu4HI    tru9I
 ahaII/bsaHI                        sfaNI     aciI              aciI  maeII         csp6I   bslI   aciI      mseI
4301 TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATACGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG GCGCATTAAG
     ACCGCGGACT ACGCCATAAA AGAGGAATGC GTAGACACGC CATAAAGTGT GGCGTATGCA GTTTCGTTGG TATCATGCGC GGGACATCGC CGCGTAATTC

                          fnu4HI
    aciI                hinPI
  fnu4HI                hhaI/cfoI                                hinPI
  aciI                 thaI                                      hhaI/cfoI
 thaI                  fnuDII/mvnI                              haeII
 fnuDII/mvnI           bstUI                             hinPI rmaI
 bstUI                 bsh1236I      aciI                hhaI/cfoI    bsrBI
 bsh1236I       maeIII  bbvI maeIII                     haeII  maeI   aciI         mboII                 maeII
4401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC
     GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA GCGGTGCAAG
```

FIG. 10N

```
                                  nlaIV
      mspI                       hgiJII                                    mnlI
      hpaII                      bsp1286                               nlaIV
     naeI                        bmyI                                  hgiCI
     cfr10I           aluI       banII         nlaIV                   banI   taqI                       hphI
4501 GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TTGGGTGATG
     CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA AACCCACTAC

         maeII    haeIII/palI
        draIII   sau96I                                     maeII  pleI         tru9I     pleI
        bsaAI    asuI                                      drdI   hinfI maeII   mseI      hinfI          bsrI
4601 GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC
     CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG

                                                                                                            thaI
                                                                                                            fnuDII/mvnI
                                                                                                            bstUI
                                                                           tru9I                            bsh1236I
          bslI                                                             mseI              tru9I       tru9I
         bslI   avaI                                               haeIII/palI        aluI   mseI   apoI mseI   apoI
4701 ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
     TGAGTTGGGA TAGAGCCCGA TAAGAAAACT AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

                                            hgiAI/aspHI
                                            bsp1286
                       maeII                bsiHKAI                                                          tth111I/aspI
                    tru9I                   bmyI    ddeI                   aciI                              maeIII
        tru9I       mseI                    apaLI/snoI  rsaI              fnu4HI      tru9I                maeII
        mseI     sspI  psp1406I             alw44I/snoI csp6I          sfaNI         mseI        aciI      bsaAI bsrI
4801 AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA
     TTAAAATTGT TTTATAATTG CAAATGTTAA AATACCACGT GAGAGTCATG TTAGACGAGA CTACGGCGTA TCAATTCGGT TGAGGCGATA GCGATGCACT

                                                     hinPI
                                                     hhaI/cfoI               mspI
                                                    thaI                     hpaII
              hinPI                                 fnuDII/mvnI             scrFI                             bsmAI
           fnu4HI                                   bstUI                   nciI     aciI                    esp3I
           bbvI                            nspBII   bsh1236I                dsaV sfaNI                      bslI
      nlaIII  hhaI/cfoI    aciI            aciI    hgaI       drdI          cauII fokI            aluI maeIII
4901 CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
     GACCCAGTAC CGACGCGGGG CTGTGGGCGG TTGTGGGCGA CTGCGCGGGA CTGCCCGAAC AGACGAGGGC CGTAGGCGAA TGTCTGTTCG ACACTGGCAG
```

FIG. 100

```
                                                         thaI
                                                         fnuDII/mvnI
                                                         bstUI
                                                         bsh1236I
     scrFI                                              hinPI
     ncìI                                               hhaI/cfoI                        mnlI
     mspI      nspI                                     thaI                           haeIII/palI
     hpaII fnu4HI                                       fnuDII/mvnI      mboII         sau96I
     dsaV aluI nspHI                                    bstUI mnlI       bpuAI         asuI
     cauII bbvI nlaIII   mnlI    hphI       hphI        bsh1236I         bbsI         eco0109I/draII
5001 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT
     AGGCCCTCGA CGTACACAGT CTCCAAAAGT GGCAGTAGTG GCTTTGCGCG CTCCGTCATA AGAACTTCTG CTTTCCCGGA GCACTATGCG GATAAAAATA

                                                                      nlaIV
                                                                     aciI
                                                                    thaI
                                                                    fnuDII/mvnI
                                        maeII                       bstUI
               nlaIII                  hinlI/acyI                   bsh1236I
       tru9I rcaI                      ahaII/bsaHI                 hinPI
       mseI  bspHI                ddeI aatII                       hhaI/cfoI
5101 AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC
     TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA GCCCCTTTAC ACGCGCCTTG GGGATAAACA AATAAAAAGA TTTATGTAAG

                  rcaI
                  bspHI
               bsrBI   bsmAI                                       mboII
               aciI nlaIII                             sspI        earI/ksp632I
5201 AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
     TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA

                                                                                           hgiAI/aspHI
                                                                                           bsp1286
                                                                                 sau3AI    bsiHKAI
                                                                                 mboI/ndeII[dam-]
                                                                                 dpnI[dam+] bmyI
                                                                                 dpnII[dam-]
              fnu4HI                                                        eco57I          apaLI/snoI
              aciI                      hphI          hphI             sfaNI mboII[dam-]    alw44I/snoI  maeIII
5301 CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA
     GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT
```

FIG. 10P

```
            sau3AI
            mboI/ndeII[dam-]    sau3AI
            dpnI[dam+]          mboI/ndeII[dam-]
            dpnII[dam-]         dpnI[dam+]                    psp1406I          hgiAI/aspHI            aciI
           bstYI/xhoII          dpnII[dam-]                  xmnI              bsp1286   tru9I        thaI
        bsrI          nspBII    alwI[dam-]                   asp700            bsiHKAI   mseI         fnuDII/mvnI
    taqI   alwI[dam-]  aciI    bstYI/xhoII               mboII maeII          bmyI     ahaIII/draI    bstUI
                                                                                                      bsh1236I
                                                                                                     hinPI
                                                                                                     hhaI/cfoI
5401 TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
     AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA

                scrFI
                nciI
                mspI
                hpaII
                dsaV
                cauII
              hinlI/acyI                                                          rsaI
              hgaI                    aciI                                        csp6I  bsrI
              ahaII/bsaHI   bcgI   mcrI fnu4HI        ddeI                       scaI hphI   maeIII     sfaNI
5501 ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT
     TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA

                                                                                                sau96I
                                                                                                avaII
                                                                                         sau3AI asuI
                                                                   haeIII/palI           mboI/ndeII[dam-]
                                                                   eaeI                  dpnI[dam+]
                                                                   cfrI                  dpnII[dam-]
                                      fnu4HI                       fnu4HI               pvuI/bspCI
      fokI  nlaIII                    bbvI       nlaIII            aciI                 mcrI  mnlI
5601 ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG
     TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC

                                  nlaIII
                               sau3AI maeIII                   mspI
                               mboI/ndeII[dam-]    sau3AI   nlaIV
                               dpnI[dam+]          mboI/ndeII[dam-]
                              alwI[dam-]           dpnI[dam+]   hpaII
     aluI  aciI            nlaIII dpnII[dam-]      dpnII[dam-] bsaWI aluI                               maeIII
5701 AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
     TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG
```

FIG. 10Q

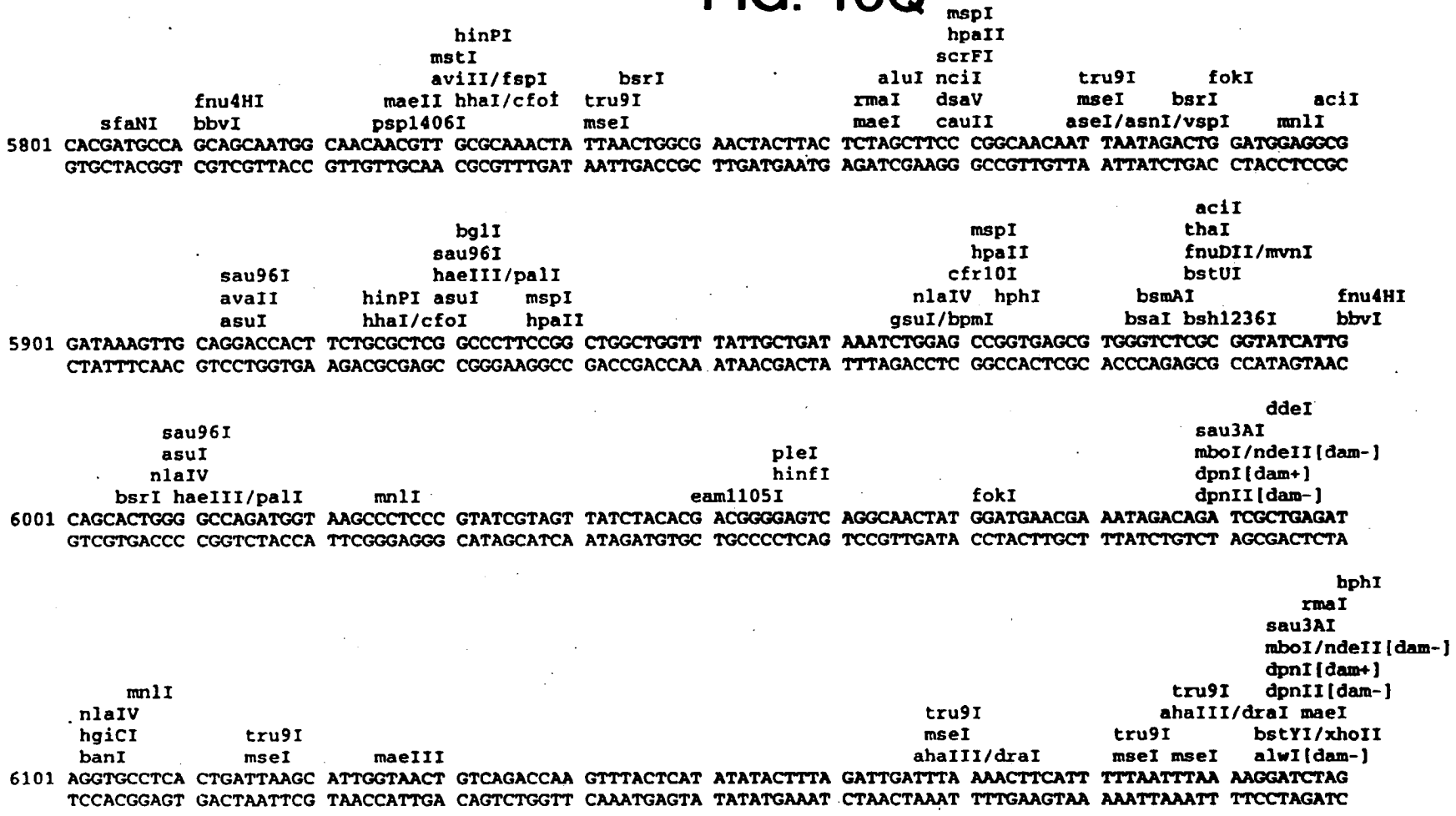
mspI
hinPI
hpaII
mstI
scrFI
aviII/fspI
bsrI
aluI nciI
tru9I
fokI
fnu4HI
maeII hhaI/cfoI
tru9I
rmaI
dsaV
mseI
bsrI
aciI
sfaNI
bbvI
psp1406I
mseI
maeI
cauII
aseI/asnI/vspI
mnlI
5801 CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG
GTGCTACGGT CGTCGTTACC GTTGTTGCAA CGCGTTTGAT AATTGACCGC TTGATGAATG AGATCGAAGG GCCGTTGTTA ATTATCTGAC CTACCTCCGC
aciI
bglI
mspI
thaI
sau96I
hpaII
fnuDII/mvnI
sau96I
haeIII/palI
cfr10I
bstUI
avaII
hinPI asuI
mspI
nlaIV hphI
bsmAI
fnu4HI
asuI
hhaI/cfoI
hpaII
gsuI/bpmI
bsaI bsh1236I
bbvI
5901 GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG
CTATTTCAAC GTCCTGGTGA AGACGCGAGC CGGGAAGGCC GACCGACCAA ATAACGACTA TTTAGACCTC GGCCACTCGC ACCCAGAGCG CCATAGTAAC
ddeI
sau96I
sau3AI
asuI
pleI
mboI/ndeII[dam-]
nlaIV
hinfI
dpnI[dam+]
bsrI haeIII/palI
mnlI
eam1105I
fokI
dpnII[dam-]
6001 CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT
GTCGTGACCC CGGTCTACCA TTCGGGAGGG CATAGCATCA ATAGATGTGC TGCCCCTCAG TCCGTTGATA CCTACTTGCT TTATCTGTCT AGCGACTCTA
hphI
rmaI
sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
mnlI
tru9I
dpnII[dam-]
nlaIV
tru9I
ahaIII/draI maeI
hgiCI
tru9I
mseI
tru9I
bstYI/xhoII
banI
mseI
maeIII
ahaIII/draI
mseI mseI
alwI[dam-]
6101 AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG
TCCACGGAGT GACTAATTCG TAACCATTGA CAGTCTGGTT CAAATGAGTA TATATGAAAT CTAACTAAAT TTTGAAGTAA AAATTAAATT TTCCTAGATC

FIG. 10R

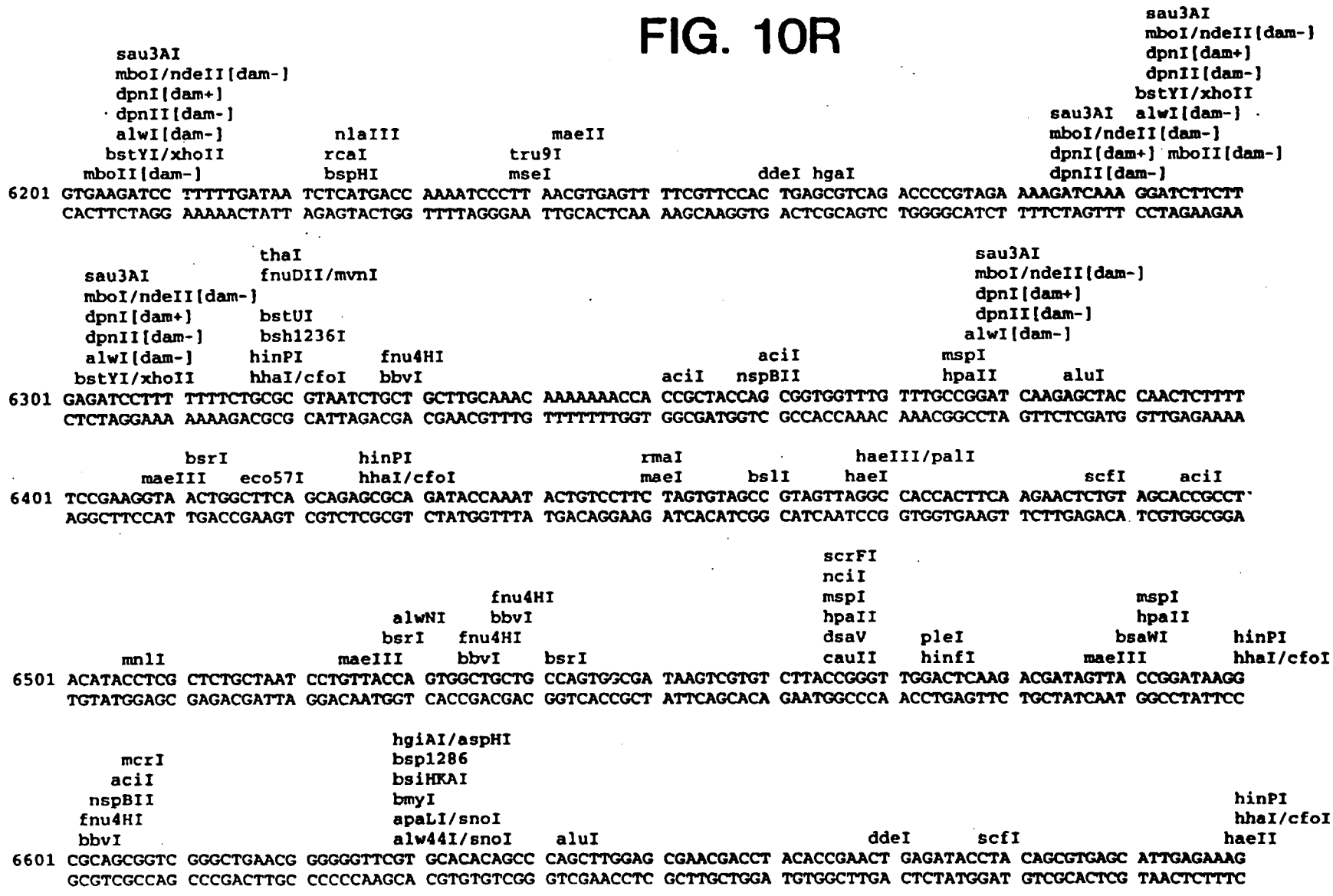

FIG. 10S

```
                                                                                              scrFI
                                                                                              mvaI          scrFI
                                                                                              ecoRII  mvaI
                                             mspI                                             dsaV    ecoRII
                                             hpaII                                            bstNI         dsaV
                                             bslI    fnu4HI                 hinPI  mnlI       bsaJI         bstNI
                                aciI        bsaWI    aciI                   hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]
6701 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
     GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC

                                                                                                                fnu4HI
                                                                                                                aciI
                                                                                                               thaI
                                                                                                               fnuDII/mvnI
                                                     taqI                           nlaIV                      bstUI
                                    mnlI  drdI    hgaI           sfaNI            aciI                         bsh1236I
6801 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG
     ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGTTGC

                         haeIII/palI
                      scrFI
                      mvaI
                      ecoRII
                      dsaV                    nlaIII
                      bstNI bslI                   nspI
        bslI          apyI[dcm+]      haeIII/palI  nspHI                      tfiI
      haeIII/palI nlaIV haeI        haeI           aflIII                     hinfI                    aciI
6901 CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
     GCCGGAAAAA TGCCAAGGAC CGGAAAACGA CCGGAAAACG AGTGTACAAG AAAGGACGCA ATAGGGGACT AAGACACCTA TTGGCATAAT GGCGGAAACT
```

FIG. 10T

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                                                                                                                     thaI
                                                                                                                     fnuDII/mvnI
                                                                                                                     bstUI
                                                                                                                     bsh1236I
                                                                                                                    hinPI
                                                                                                                    hhaI/cfoI
                                                                                                                   thaI
                                                                                         hinPI                     fnuDII/mvnI
                         fnu4HI                fnu4HI                               sapI hhaI/cfoI                 bstUI
                         bbvI                  bbvI  pleI                           mboII                          bsh1236I
                  bsrBI  aciI                hinPI   hinfI                          earI/ksp632I            mnlI    bslI
        aluI      aciI  fnu4HI       mcrI    hhaI/cfoI              mnlI    aciI    haeII              aciI         aciI
7001 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG
     CACTCGACTA TGGCGAGCGG CGTCGGCTTG CTGGCTCGCG TCGCTCAGTC ACTCGCTCCT TCGCCTTCTC GCGGGTTATG CGTTTGGCGG AGAGGGGCGC

                  tru9I    aluI
       haeIII/palI         pvuII                                                        tru9I                  nlaIV
      eaeI tfiI   mseI     nspBII                                        hinPI          mseI      maeIII       hgiCI
      cfrI hinfI aseI/asnI/vspI                  bsrI      aciI          hhaI/cfoI  aseI/asnI/vspI mnlI        banI
7101 CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG
     GCAACCGGCT AAGTAATTAG GTCGACCGTG CTGTCCAAAG GGCTGACCTT TCGCCCGTCA CTCGCGTTGC GTTAATTACA CTCAATGGAG TGAGTAATCC

       scrFI
       mvaI
       ecoRII
       dsaV
       bstNI
       apyI(dcm+)                                          aciI                                                xmnI
      bsaJI                    mspI                       bsrBI                                                asp700
                               hpaII                                                     aluI       nlaIII
7201 CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA
     GTGGGGTCCG AAATGTGAAA TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT GTGTCCTTTG TCGATACTGG TACTAATGCT

      tru9I
      mseI
     aseI/asnI/vspI
7301 ATTAA
     TAATT

>length: 7305
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/09576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/67 C12N15/85 C12N9/72 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DNA CLONING, VOLUME III, EDITED BY D.M. GLOVER, 1987 IRL PRESS, OXFORD, GB;, pages 189-212, A.M.C. BROWN AND M.R.D. SCOTT 'Retroviral vectors'	1-3,7,8
Y	see page 192, line 7 - page 196, line 5; figures 2,3	5,6, 9-12, 16-21
	--- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1995	08.12.95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Hornig, H

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CELL, vol. 37, no. 3, July 1984 CELL PRESS,CAMBRIDGE,MA,US;, pages 1053-1062, C.L. CEPKO ET AL. 'Construction and applications of a highly transmissible murine retrovirus shuttle vector' cited in the application	1-3,7,8
Y	pZIP-Neo SV(B)1 see figure 1	5,6, 9-12, 16-21

Y	MOL. CELL. BIOL., vol. 5, no. 3, March 1985 ASM WASHINGTON, DC,US, pages 431-437, A.D. MILLER ET AL. 'Generation of helper-free amphotrophic retroviruses that tranduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene' see page 432, right column, line 5 - page 436, right column, line 7; figure 1	5,6, 9-12, 16-21

Y	WO,A,94 05784 (US) 17 March 1994 see the whole document	5,6, 9-12, 16-21

Y	EP,A,0 215 548 (ZYMOGENETICS INC ;UNIV WASHINGTON (US)) 25 March 1987 see the whole document	5,6, 9-12, 16-21

A	WO,A,92 17566 (GENENTECH INC) 15 October 1992 cited in the application see the whole document	1-21

A	WO,A,90 12025 (UNIV LELAND STANFORD JUNIOR) 18 October 1990 cited in the application see the whole document	1-21

A	EP,A,0 260 148 (GENENTECH INC) 16 March 1988 see the whole document	1-21

A	EP,A,0 160 457 (GENENTECH INC) 6 November 1985 cited in the application see the whole document	1-21

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 86, February 1989 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 1041-1045, M. VIVAUD ET AL. 'A 5' splice-region G-C mutation in exon 1 of the human beta-globin gene inhibits pre-mRNA splicing: A mechanism for beta+-thalassemia' see the whole document	1-4

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 95/09576

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		JP-A-	6040942	15-02-94
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